TECHNOLOGIES TO MAINTAIN BIOLOGICAL DIVERSITY

Volume II--Contract Papers

Part B: Animal Technologies

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6. Constraints to International Exchange of Animal Germplasm
   (William M. Moulton)

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Contract Paper No. 1

Status and Trends of Wild Animal Diversity

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STATUS AND TRENDS OF WILD ANIMAL DIVERSITY

Prepared for the Office of Technology Assessment

by

Nathan R. Flesness

1985

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ABSTRACT

Conservation, or the claimed lack thereof, is a major topic of discussion in many public policy debates. The key issue is to what extent the patrimony of future generations of humankind is being denied them by this generation's actions; would a good accountant find that we are handling "generational equity" fairly?

For many kinds of animal species, there is a tremendous lack of direct, hard information. Most species of animals (notably invertebrates) have never even been scientifically described or named; since they have therefore never been "inventoried", their loss goes unnoticed.

What historical data is available (for terrestrial vertebrates) shows a statistically significant increase in recorded extinction rates around 1825. It is not clear if this represents increased knowledge, or actually increased extinctions.

Recent historically recorded extinction rates are higher than anything comparable in the fossil record. Even the catastrophic periods in paleontological history show lower average world-wide vertebrate species loss rates. Today's extinction rates are ten to a thousand-fold higher than "normal".

Estimates of unrecorded species loss rates have to be made from habitat loss data (itself hard to come by). The best calculation possible is somewhat more optimistic than earlier widely-publicized figures, but the results still indicate that current habitat destruction is costing something like one species per hour (including animals and plants).

The latest gene-transfer technology magnifies the expected utilitarian value of wild animal genes considerably. Therefore losses of such species and genes are more clearly costly than before.

Very little is being invested to create more accurate monitoring of wild animal species diversity, even though some of the needed mechanisms and institutions already exist, and urgent recommendations on priority, strategy and tactics have already been made by prestigious scientific study groups. Better monitoring, which even conservation-critics agree is desirable, could be done for a few million dollars annually.

Very little is being done to reduce the damage, or even study methods for amelioration.

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ECONOMIC AND ECOLOGICAL DEPRESSION - A PARALLEL

Each generation of humans inherits material goods, genes (species), and ideas from the preceeding one. By far the easiest of these to replace when lost or wasted are the material goods (Hardin, 1974).

Surviving species, and the genes within them, are the best results of uncountable numbers of blind trial-and-error experiments that have been performed since life began on earth 3.5 or more billion years ago. The billions, perhaps trillions, of surviving genes are the ones that helped their organisms function well enough to meet the competition. The millions of extant species are those that have worked well enough at making a living and reproducing in the natural economy that they, not their competitors, survived.

It is a commonplace that we humans remain dependent on the natural economy. Our own economic activities largely assume clean water, breathable air, moderate weather, farmable soil, burnable fuels, harvestable timber, edible fish and game. Our food comes primarily from a few species of animals and plants which we have domesticated from wild stock (Oldfield, 1984). These things are not "natural", but rather are the specific results of an extraordinary and poorly understood natural economy - on which our own human economy is critically based.

It is also a commonplace that species are irretrievably vanishing from the natural economy. Similar to when companies vanish from our own economy, "going-out-of-business" events by species produce dislocations. Sometimes these are severe, sometimes undetectable, but our level of ignorance is so high that evaluation is hard to do. Raw materials, previously consumed, may pile up in inconvenient quantity, and an honest and appreciated product, rarely noticed much, suddenly is no longer available. Others may suddenly be "unemployed" now that a specific enterprise is missing, either directly or from a "ripple" effect through dependent economic activities.

Some of this, too, is natural in either economy. Better ways of doing things come along, old gives way to new, and the change may be progress. Companies go out of business, and new ones appear with higher efficiency, novel method of organization and production. Species too have been going extinct for at least hundreds of millions of years, and new ones have appeared through speciation.

There is a need for a balance though. A mere doubling of the business failure rate was the Great Depression (U.S.B.C. 1961). Such business imbalance means bad times for years, perhaps even a decades, until conditions can be changed, new ventures started, and balance restored.

The natural situation is parallel. If the *rate* of extinction of the economic concerns, or species, is significantly higher than the replacement, or speciation, rate, a "depression" will certainly result. This depression would show itself as reduced biological activity, and as various kinds of dislocations. However, since the fastest presumed vertebrate speciation event is about as long as all of recorded human history (4,000+ years), recovery of the natural economy would take a radically longer time than is traditional for the human economy.

Therefore, knowing the rate of disappearance of species, and of the genetic diversity within them, is even more vital for human affairs than knowing the business failure rate.

TAXONOMIC LEVELS

Evaluation of loss and retention of wild animal genetic diversity would ideally involve assessing loss and survival of populations with significant genetic uniqueness. This should be without regard to their previous taxonomic classification, as such classification is almost always based on very limited morphological and biogeographical data.

Very regrettably, this approach is totally impossible with present data. It would need detailed, population-level range information for all species, both presently and in the past. It would also need genetic study of all populations, to assess which are genetically unique. Current research activities in evolutionary biology and molecular systematics are just beginning to produce such data for an extremely small proportion of species and populations (e.g. Dillon, 1984).

At the next higher level of resolution, loss of subspecies may represent significant erosion of within-species genetic diversity, or alternatively could represent as little as the loss of a single gene whose morphological effects caused its geographically localized carriers to be distinguishable. Since most species, let alone described subspecies, have not been genetically characterized, it is impossible to evaluate the genetic meaning of recorded extinctions of subspecies.

Species taxonomic boundaries are somewhat better-defined, and since they are fewer in number than their subunits, have benefitted from relatively more study. Indeed, recent compilers of extant species lists (Honacki et. al., 1982; AOU 1983) and endangered species lists (Thornback and Jenkins, 1982; Collar and Stuart, 1985) have completely omitted subspecies. The omission is on grounds priority, and recognition of subspecies uncertain meaning in most individual cases.

Therefore, historically recorded extinctions analyzed immediately below are limited to those taxa described as species, with the added restrictions that physical remains must exist, and that history (as opposed to paleontology) must provide at least an approximate date. There is a large and growing body of evidence that pre-historical-record extinctions linked with human activity are much more numerous than had been believed until recently. Archeological and paleontological investigations of many oceanic islands are just beginning (Diamond, 1982; Harrison, 1984; Olson and James, 1984). Those that have been conducted show a surprising number of previously unknown extinct animal forms linked with human occupation.

RATE OF SPECIES DISAPPEARANCE I. HOW MANY ARE THERE?

To know that something is gone, you have to know it was once there. This is a seemingly trivial point, but it represents a large obstacle in measurement of extinction rates. To calculate this rate for comparison with species origination rates, one would first like to know how many species there are.

For wild animal species there is at least 10-fold uncertainty about the answer to this question of numbers. Estimates range from 2.5 million to 10 million species (Sabrosky, 1953; Raven, 1974), on to considerably more than 30 million (Erwin, 1982). Of this uncertainly large number, perhaps 1.2 million (Huevelmans, 1983) to 1.6 million (Myers, 1979) have been scientifically described (generally just measurements and appearance, not ecology, behavior, etc.).

These estimates yield a range of 4 - 40% as the proportion of the world's current animal species ever studied by a biologist. Birds and mammals are the best-known groups by far, but a new species (assigned to a new genus) of bird was found just eleven years ago in Hawaii (Casey and Jacobi, 1974), a new species of pig in Paraguay (the largest living pig) just ten years ago (Wetzel et.al. 1975), and many bats and small rodents remain undescribed.

. The rate of discovery of new animal species (including invertebrates) apparently peaked at perhaps 12,000 per year between 1900 - 1950, and has now dropped to an estimated 5,200 per year. Mammal and bird new species discovery rates have dropped to about 11 and 3 species per year, respectively (Huevelmans, 1983). Diamond (1985) provides a chronological picture of mammalian genus and avian species discovery rates in recent years, and notes that rates have declined to a low plateau. Most newly discovered mammals and birds are cryptic, small, or very restricted in range.

Faced with so much uncertainty in the number of species at risk of loss, there are two different approaches used to estimate species extinction rates.

HISTORICALLY RECORDED EXTINCTIONS - TERRA COGNITA

The most concrete approach is to tabulate the names and dates of species that are known to have gone extinct within recorded history. The constraints of name and date are surprisingly limiting. Species did not even begin to have consistent names until Linnaeus (Systema Naturae, 1758). A century later, only 126 years ago, humans first understood something of how species originate (Darwin, Origin of Species, 1859). What recorded history is available is essentially that of the West, and most of the record is based on explorer's accounts, which are less than comprehensive or systematic.

The historical record of species extinctions runs back to about 1600, with many biases. It logically takes at least two visits to a place (one for discovery, one to note loss) to generate a recorded extinction. The English, whose explorations placed some emphasis on natural history, began their first really extensive voyages in the period 1550-1600 (Hakluyt, 1972).

The fact that only two "certain" recorded and approximately dated insect extinctions (Xerces Blue butterfly, *Glaucopsyche xerces*; Tobias' Caddis-fly, *Hydropsyche tobiasi*) were encountered (Opler, 1976; Pyle et. al. 1981; Wells et. al. 1983) in this literature review shows some of the bias. Insects outnumber all other forms of life, and especially in the tropics are very vulnerable to extinction when a chain of obligate mutualist species is broken (Gilbert, 1975). Many other described invertebrate species are believed extinct, but survey efforts were and are so incomplete that the extinction dates cannot be estimated reasonably (see especially the snails and mussels listed in Opler, 1976; Wells et. al. 1983).

Large terrestrial and avian vertebrates were presumably much more likely to have their presence, and then later absence, noted. Small forms were less likely noticed, and marine forms were and are nearly impossible to census. If you can't be counted, you can't be counted as zero - so actual recording of extinctions in such species is improbable.

A number of reviewers have assembled or summarized what documented extinction reports can be found (Allen, 1942; Harper, 1945; Greenway, 1967; Ziswiler, 1967; Goodwin and Goodwin, 1973; King, 1979; Day, 1981, Thornback and Jenkins, 1982; Wells et. al. 1983; Nillson, 1983, Diamond, 1984). These compilations and reviews provide the principal data available on historically recorded extinctions.

Analysis of the data presented by these sources must be done with care. Some biases were discussed above. Additionally, summary presentations often mix extinctions of subspecies and species together (especially the older sources), and may include taxa described only from "subfossil" remains or potentially fanciful traveler's accounts. Further, some writers (e.g. Opler, 1976) use "extinction" without clarification of scope, conceivably meaning either extinct within national boundaries or extinct globally - a potentially serious ambiguity.

As an example of previous mis-statements about extinction in public policy discussion, Harrington and Fisher (1982, p124) state:

> "In the United States, over 500 species (emphasis added) are known to have become extinct since the year 1600, an average of one to two per year."

They cite Opler. Opler actually said (1976, p.30):

> "...North America...since the arrival of the Puritans at Plymouth Rock in 1620, over 500 species and subspecies (emphasis added) of native animals have become extinct."

Opler's Table 1 (pp.32-33) lists 112 full species (54 of which are snails and mussels) listed as extinct "within the U.S.". It is unclear whether these species are thought to be extinct outside the U.S. as well, though 65 of the 112 species are apparently Hawaiian endemics and therefore are presumably globally extinct. By dropping the words "and subspecies", Harrington and Fisher exaggerated the rate given by their source by a factor of 3- to 6-fold; 112 species in 376 years is 0.3 species per year instead of the one to two per year they state.

THE HISTORICAL PATTERN: VERTEBRATE SPECIES EXTINCTIONS

The overall historical pattern for vertebrate species extinctions is presented below as Figure 1. This bar graph is a conservative view, for the reasons presented above. The most noteworthy feature seems to be the dramatic dichotomy between the left-hand side (1600-1825) and the right-hand side (1826-1975). Apparent mean extinction rates are 0.124 species/year and 0.767 species/year respectively, a six-fold increase for the period since 1825. This difference is statistically significant ($p<.005$ by t-test).

Available information does not allow resolving whether the apparent increased extinction rate of the last 160 years is real, or is merely due to less complete knowledge and records of extinctions in earlier times.

THE VERY RECENT PATTERN

A further important question concerns whether the recently higher level of ecological consciousness, environmentally protective legislation and treaties has had any measurable impact on known vertebrate extinction rates. The most recently completed 25-year interval (1951-1975) already includes 14 recorded vertebrate animal species extinctions, for a rate of 0.56 species/year.

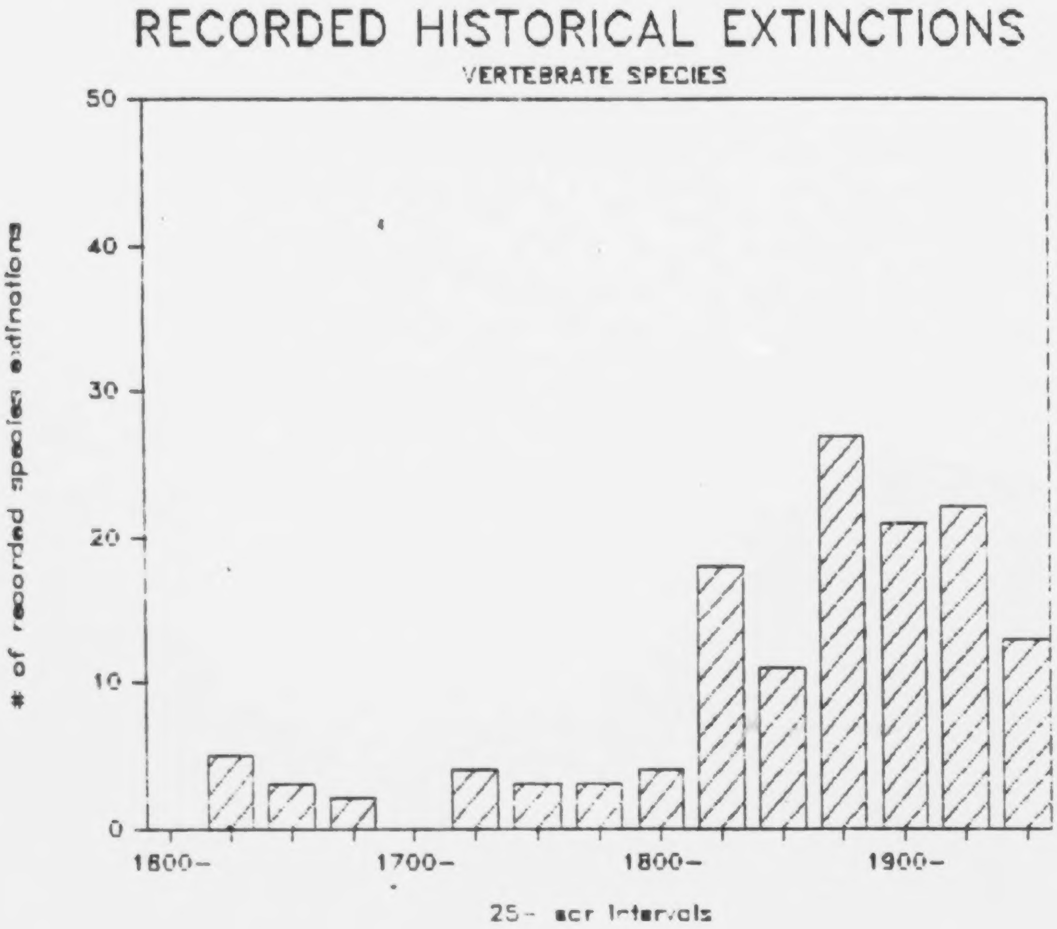

RECORDED HISTORICAL EXTINCTIONS
VERTEBRATE SPECIES
# of recorded species extinctions
50
40
30
20
10
0
1600-
1700-
1800-
1900-
25- ycr Intervals

Figure 1

This is not significantly different than the .767 species/year rate since 1825 (it would have to be quite different to be statistically significant with sample sizes this small). It is a little smaller, but it is also incomplete in the special sense that tabulation of extinctions in this time interval will take many more years.

There may be a lag of some decades before extinctions in any one period can be evaluated. Given the difficulties of finding rare organisms, any rational criteria for extinction has to include some time lag for evaluation. The Convention on International Trade in Endangered Species (C.I.T.E.S.) criteria is that no individuals have been seen for 50 years.

Under these CITES criteria, adopted widely by others including most of the IUCN/SSC/Species Conservation Monitoring Unit's Red Data Books, no animals would be counted as having gone extinct in the interval 1975-1985 until the period 2025-2035! This lag time causes all kinds of difficulties for policy decisions, but was presumably deemed necessary because the very limited resources for evaluating wild species status means that survivors may be simply overlooked for long periods. In some regions and for some taxa (e.g. U.S. terrestrial vertebrates), more active monitoring efforts may sometimes determine population status before 50 years have passed.

As an example of the time lag, the U.S. Fish and Wildlife Service is now conducting a status review for the Ivory-Billed Woodpecker, whose last accepted sighting was in the early 1950's (Anon, 1985). If this species is confirmed extinct, the extinction date will most likely be estimated in the interval 1950-1975.

Though we are only ten years into the next quarter-century, it is obvious that there will be at least enough extinctions to maintain the pattern. Just one island, Guam, has lost five endemic bird species since 1980 (Derrickson, pers. comm.), though three may be maintained in zoological captive breeding programs.

It is also worth noting that all but one of the vertebrate extinctions so far identified between 1950 and 1975 took place in the U.S. or Caribbean. This suggests that whereas monitoring may be better, extinctions are still occurring at a significant rate within the U.S. and immediately surrounding areas. This may also be interpreted as suggestive evidence for the very poor monitoring of much of the rest of the world.

As one check on the general validity of this historical pattern, the larger, less restrictively constructed list of vertebrate species extinctions compiled in the Endangered Species Handbook (Nillson, 1983, for the Animal Welfare Institute) was examined. This list includes 191 extinctions, instead of the 139 evaluated above. The criteria for inclusion are less stringent, adding 52 additional extinctions. Yet a third recent review (Diamond, 1984), using a couple of unpublished sources not yet available to this reviewer, arrived at a total of 151 species extinctions for mammals and birds since 1600. Since Diamond did not present the actual data, comparison with his list is not possible here.

However, the pattern of extinction events reported by Nillson is very similar to that shown in Figure 1, with a dramatic dichotomy between periods before and after 1825, and an apparently shorter bar (lower rate) for 1950-1974. Again, t-test of the pre- and post-1825 rates (from Nillson's compilation) shows that they are significantly different ($p = .011$). Again, t-test of the 1955-1974 data against the post-1825 rate shows that they are not significantly different ($p = .50$).

Such an elementary analysis of apparent rate differences is somewhat simple-minded. Far more sophisticated analytical methodologies are being developed for pre-historic extinction rate measurement (e.g. Conroy and Nichols, 1984), but their extension and application to historical data is a subject for research.

UNRECORDED EXTINCTIONS - TERRA INCOGNITA

Even more difficult than assessing known-extinction rates, is assessing the rates of unknown extinctions. Most biologists live and work in the temperate zone, whereas most species are in the tropics. An obvious consequence is that we have much less extinction data for the most species-rich parts of the world.

What data does exist relates primarily to habitat reduction. One-third to one-half of the world's moist tropical forests have been cut (Myers, 1980; Lanly 1982; Haas, 1983, Melillo et. al. 1985; see also Lugo and Brown, 1982), similar to the one-half of total wetland habitat drained and destroyed within the U.S. (Tiner, 1984). However, these events are believed to have quite different consequences.

Temperate ecosystems were dramatically disturbed by Pleistocene glaciations over the last 3 million years, and also by loss of large land animal forms (Martin, 1984). For these and perhaps other reasons, many of the surviving species are relatively widespread generalists. Though it has not been well studied, subsequent species loss among members of the North American prairie ecosystem may be modest, even though perhaps 98.4% of the habitat has been destroyed by humans in historic time (Myers, 1979, p.5). The loss of diversity at the population or subspecies level was no doubt very high, but for temperate prairies and wetlands, a high fraction of the species are widespread in significant numbers and thus survive in small numbers somewhere until almost the very end of the habitat.

In contrast, tropical ecosystems may have evolved in a history of less disturbance (though see discussion in Simberloff, In Press) at any rate they appear to include far more specialized and tightly interdependent forms (see e.g. Gilbert and Raven, 1975). Species diversity of trees, insects, frogs, butterflies, etc. is much higher in the tropics - but individual species densities are much lower (Elton, 1975; NRC, 1980; Lovejoy and Brash, 1984). Endemism (restriction of species to a local area) is also higher (Haffer, 1969; Terborgh and Winter, 1983).

The consequences of these differences between temperate and tropical communities all go in the same direction. The rate of loss of species from tropical habitats is expected to be higher than that of temperate habitats for equivalent proportional reductions in area.

The key question is how much higher? Species-area relationships are an area of active research, but many of the models so far developed are not very predictive (Boecklen and Gotelli, 1984). Lovejoy (in Barney, 1980-1981 [Global 2000 Report to the President]) developed a range of possible curves relating proportion of species extinct to proportion habitat loss. The results using any of his curves are dramatic - i.e. hundreds of thousands of species extinctions. The two difficulties (discussed by Simon and Wildavsky, 1984) are 1) are the deforestation estimates reasonable, and 2) might the real curve be far more optimistic (concave) than Lovejoy's?

Simberloff (In Press), using a somewhat more rigorous species-area model, plus newer remote-sensing deforestation estimates (Lanly, 1982) modelled the expected loss of species of neotropical plants and Amazonian birds. He states that his results are somewhat robust - changes in exact parameter values have relatively little effect on the results.

His conclusions are that in the next 15 years the "neotropical rain forest will have been sufficiently reduced to cause massive extinction at some time in the future of both birds and plants". If logging unexpectedly stopped in 15 years, he predicts a resulting loss of 15,000 plant species and about 100 bird species. He further projects that with logging continuing until all but protected areas were cut (all but about 1%, analogous to the U.S. record with prairie preservation), 66% of neotropical plant species and 69% of Amazon bird species will go extinct as a result.

Simberloff's modelling provides at least a way to evaluate the less formally derived Lovejoy curves. Simberloff's values (his Table 1) suggest that Lovejoy's most optimistic curve, "E", was the closest - and that 5% to 15% less extinctions are projected than Lovejoy's most optimistic estimate for a given level of logging (see Figure 2 herein). For example, instead of Lovejoy's projection of 375,000 to 1,250,000 extinct species at 67% logging of tropical forests (based on his intermediate curve, "C"), application of Simberloff's model suggests that "only" half as many, 187,000 to 625,000, would go extinct.

That these two projection efforts differ by a factor of two is, in a certain sense, trivial. As Simberloff stresses, our ignorance of tropical biology is nearly complete, and our estimates necessarily rough. Extinction numbers anywhere around this order of magnitude can be compared only with the few massive extinction events of the fossil record.

Species Extinctions vs. Habitat Loss

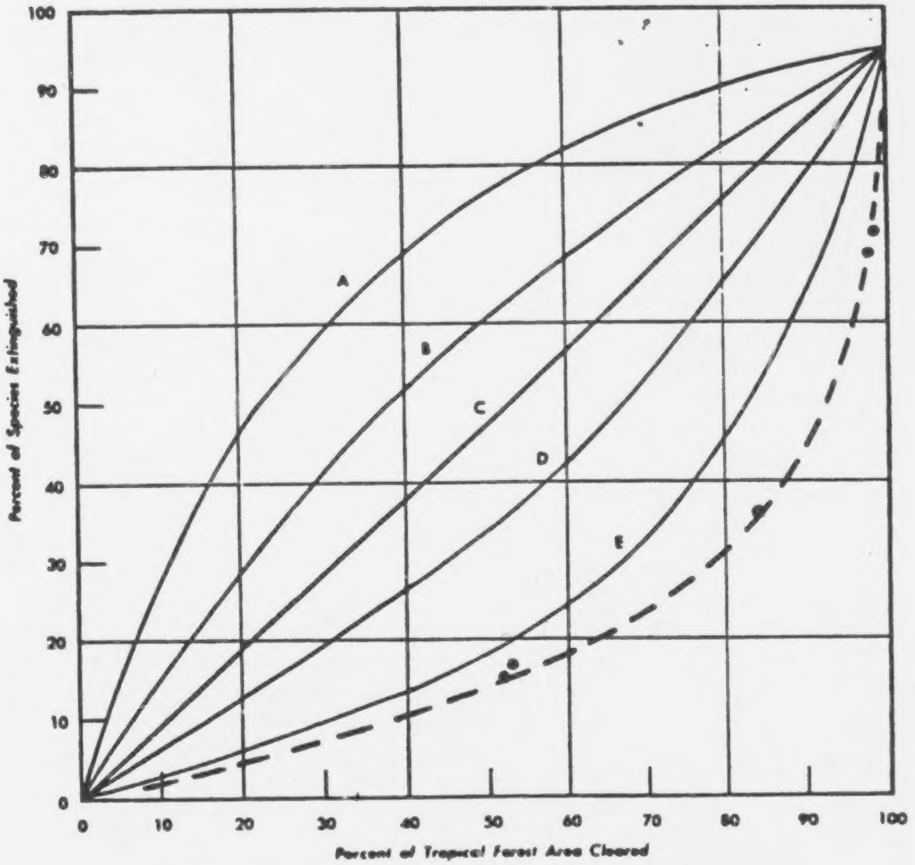


Figure 13-8. Loss of species through clearing of tropical forest areas—five projections.

Original Figure from Lovejoy (1980/81) Global 2000 Report to the President

Dashed line based on Simberloff, 1985 data (from model)

Figure 2

COMPARISONS TO THE PALEONTOLOGICAL RECORD

The accuracy and resolving power of the fossil record has been the subject of much recent analysis and debate (e.g. Stanley, 1985; Gingerich, 1985). This has been organized around the issues of "punctuated equilibria" (Eldredge and Gould, 1972) in evolution. Punctuated equilibria vs. phyletic gradualism is a debate over whether morphological change generally occurs throughout a lineage's history, or whether most change occurs as a concomitant of speciation events. Since changed morphology is how taxa are recognized in the first place, this issue has consequences for estimation of extinction rates. Schopf (1982), Stanley (1985), and Gingerich (1985) have estimated worldwide species' average durations in this context.

Schopf argues that biases in interpretation of the fossil record led to systematic overestimation of species durations, and suggests that 200,000 years is a closer figure than the 10,000,000 year estimate produced by others he cites. Importantly, the range of dispute on species' lifetime is 10 to 100-fold. Using the approximately 13,000 living species of mammals and birds, the 200,000 year species' duration figure would lead one to expect an equilibrium rate of 0.065 (mammal and bird) species extinctions per year globally. This is the highest value derivable for a "normal" extinction rate.

The one to several million year mean species duration estimates of Stanley (1985) and Gingerich (1985), would dramatically reduce the estimated "normal" figure to an expected equilibrium of 0.0013 (mammal and bird) species extinctions per year.

Webb (1969) roughly estimated that 50 - 90 land mammal species went extinct in North America each million years during the late Cenozoic (the last 3 million years). This period of time covers all of the Ice Age disturbances plus human immigration, and is widely thought to be a high-extinction period. Using his figures for the Recent running mean generic diversity, calculated as species (78 genera x 4.2 avg. species each = 327 species), and extrapolating to the 13,000 globally extant mammals and birds, yields a comparable extinction rate of 0.002 mammal and bird species per year, globally.

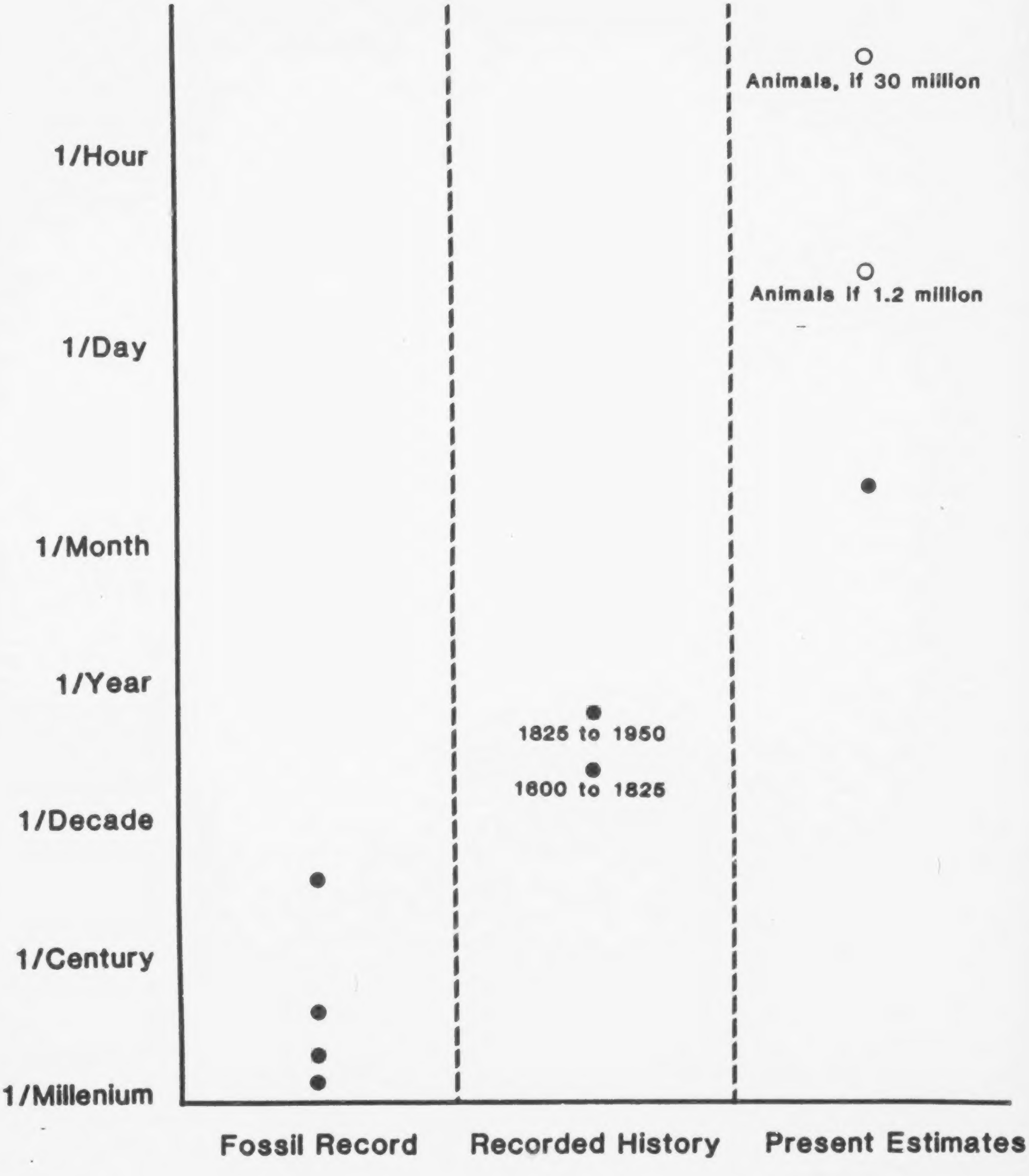
In a recent review, Webb (1984) states that the highest extinction rate in the last 10 million years was the Rancholabrean, when 40 genera were lost, and North America's large mammal fauna devastated. Graham and Lundelius (1984) provide a list of Rancholabrean extinctions at the level of species, identifying 72. Assuming that all extinctions actually were "synchronized within a few thousand years" (Webb, 1984) - using 3 for "few", one arrives at 0.024 North American mammal species extinctions per year as the peak rate - a rate so high that its explanation is the subject of long-term continued research and debate. A considerable body of work implicates man in these high extinction rates (Martin and Klein, 1984).

For Pleistocene mammals of Europe, 57% of species went extinct in a 1 million year interval (Kurten, 1968). This longer-term time interval of course could have included higher and lower rates within it. Computing what this average rate would mean today, with 13,000 mammal and bird species extant, one would expect 0.0074 species extinctions per year.

Figure 3 summarizes the various extinction rate estimates derived here - for both recorded and unrecorded extinctions. To make data derived for different taxa more comparable, the various extinction rate values are shown when applied to today's 13,000 mammal and bird species. To show the impact of these rates on the total animal fauna, two additional points are presented (with the upper and lower boundary conditions for animal species diverity).

In order to fit the wide range of values on one graph, the ordinate uses a log scale.

SPECIES EXTINCTION RATES



Solid circles show loss rates applied to 13,000 mammal and bird species presently extan

Open circles show loss rates applied to total animal fauna

Figure 3

WILD ANIMALS OF POTENTIAL VALUE FOR DOMESTICATION

No new wild animal species has been "fully" domesticated in a very long time - probably thousands of years. Most of humankind's animal protein (meat and milk), as well as still important motive power (for the Lesser Developed Countries, primarily) comes from just a dozen domesticated forms which we inherit from pre-history (Clutton-Brock, 1981; Oldfield, 1984). Such dependence on a few domestic animal species is risky, and doubtless less economically and ecologically efficient than a broader range of domesticated forms would be.

The wild species ancestral to our few domestics have considerable value as a source for needed genetic variability. One example would be disease resistance. The wild species' populations are continuously challenged by pathogens and may carry resistance or immunity to disease that may harm domestics. However, these ancestral species are typically threatened as a logical result of their initial utility.

The domestic form can usually be grown in much the same range as the wild form - but in simplified and managed habitat. Therefore humans naturally convert the wild species habitat to ranches and farms. Furthermore, wild animals remaining in the vicinity may be a competitive threat because they eat the same food and communicate the same diseases as the domestic, and so are killed or driven away (N.R.C. 1983; Oldfield, 1984). Lastly, they may be hunted for food - familiar food, since they are similar to the domestic strain. These factors were apparently involved in the cerca 1627 extinction of the Aurochs, _Bos primigenius_, the major ancestor of today's domestic cattle. The Aurochs would likely be a very useful source of cattle genes for efficiency on rough forage, hardiness and disease-resistance, were it surviving today.

Short of full domestication, another potential value for wild species - beneficial crossbreeding with existing domestic species - has had only modest application (Short, 1976). One reason is the long time period required to go through many generations of selection of desirable traits after the cross. Significantly, one such hybridization program which is continuing in North America, the buffalo-cattle crosses known as "beefalo" (Oldfield,1984), utilizes a wild species which was saved from the brink of extinction through captive breeding at the beginning of this century (Grzimek, 1972).

Oldfield (1984) and N.R.C. (1983) identify species which have considerable domestic potential either directly or through hybridization with domestic species. Many of these wild forms are endangered, some (e.g. kouprey, _Bos sauveli_) are possibly extinct. The tarpan, _Equus caballus ferus_, had such potential but was lost 135 years ago. The Przewalski horse, _Equus przewalski_, has apparently gone extinct in the wild some time after 1947, when the last capture was made, but several hundred survive in zoos. They also have potential as a source of "hardiness" genes.

Given this lackluster history in recent years, it is something of a paradox to say that wild animal species may now be of the greatest imaginable value for domestic animal husbandry purposes. The reason is the very latest technology.

During the year of this study, the first report of insertion of foreign genes into large domestic mammals was published (Hammer et. al. 1985; news report in Lovell-Badge, 1985). This accomplishment completely changes the value of genes in wild species, as it means that fertility barriers preventing cross-breeding between wild source species and domestic recipient species are no longer relevant. Investments in such efforts could now pay off in as little as one animal generation.

Given the poorly understood complexities of eukaryote development, the initial payoffs of this technological advance are likely to be from transfers of one or a few genes. Such might confer greatly improved growth characteristics (i.e. direct growth hormone levels), improved disease tolerance, etc.

With further research in molecular embryology, payoffs may be expected from "transgenesis" of major blocks or functional groups of genes. This is probably not so close at hand. However, the potential for making major shifts in the capabilities, efficiencies, and tolerance of domesticated animals seems eventually limited only by the questionable survival of wild source species and genes.

AUDITING DIVERSITY

Audits have already been called for (NRC 1978, p.96; NRC 1980, pp. 2-3 & 46-60; Raven, 1974; Raven, 1983; and even by the skeptics - Simon and Wildavsky, 1984, p.175).

The following information is needed to provide reasonably sound estimates of the rate of erosion of animal diversity and the genetic diversity this represents:

1. Rates of habitat loss, and remaining total area, by type.
2. Pattern of extinction, immigration, and persistence within and between now disjunct patches of habitat.
3. Current data on single species status, where feasible.

Habitat Status and Change Rate: This is the most critical set of data needed. Judicious application of remote (e.g. satellite) sensing and carefully designed "random block" ground truth evaluations could provide accurate and timely information on status and trends of habitat types.

Initially these censused types should be broad categories, perhaps just "virgin" forest of the major types, areas cycling through slash and burn agricultural use, areas in commercial forest, scrub, grassland, cropland, and desert. A first cut at patterns and scale of insularization (fragmentation) should also be possible. Such data is the highest priority need. Such data, and only such data, would define the current global situation and its rate of change.

In certain cases, existing databases could be used to independently check the estimates. Such databases include, for the U.S., the Department of Interior's Wetland Inventory (Eldridge, 1985).

Using available expertise (UNEP, FAO, IUCN Protected Areas Data Unit, academic and government) and available satellite data, rough answers might be feasible rather promptly and relatively cheaply. Rough answers would be better than the biased or fictitious habitat data now in common use (Robinson, 1985). A naive estimate would be two years and two million dollars to produce an initial global status report and rough world database with breakdown by major habitat type. These figures allow for a working group of ten assorted professionals, extensive travel, and computer equipment purchase.

To move so quickly would require that the group be given freedom from the usual procedures controlling travel and purchase, as well as provided international political support to arrange access to trouboled or restricted areas.

Adding finer resolution and better validation, as well as maintaining the data, monitoring and reporting annually, would add ongoing costs of like annual amount (the same staffing should be able to do a better job each year as they build on the previous database). Such annual monitoring and reporting would be essential to evaluate the directions and rates of change.

Consequences of fragmentation: We are dividing up what remains of wild habitats into small pieces, the equivalent of islands. Islands usually have reduced diversity. One one controlled study is being done to study what happens during and after the division (Lovejoy et.al, 1983).

Information from fragmentation studies of this kind is critical to deciding on reserve shape, size, and structure. Even a little more information would help a great deal (many choices for reserves will probably vanish before most such studies could run to completion). The consequences of what we don't know about this are already having major impact within the United States. Reserve and forest management decisions are now made without this information (e.g. Harris, 1984). Our children and their children will live with the consequences; hundreds of years will pass before habitat management errors can be repaired (species' extinctions are of course irreparable).

Though the time is late, performing these fragmentation experiments is cheap (about $400,000 per year each) - the world could use and well afford more than one. Demarcation and survey/census of the designated study areas prior to logging or other disturbance around them should be followed by roughly yearly survey/census work thereafter. It is likely that prairie will lose diversity differently than moist tropical forest, and that temperate forest will differ again - but we won't know unless the studyies are done. With this kind of information, more animal species diversity can be retained at less economic cost than with ignorance (Harris, 1984).

Databases on Individual Species. Establishing and maintaining databases on individually identified animals species has different purposes than habitat inventory. We have special interest in the fate of certain species, as identified above under domestication issues. We generally have more interest in easily identified, large species, with long human association and interest. Our interest is often higher for species more closely related to humankind. There is also reason to suppose that some groups are more at risk than others - that a greater relative proportion of rhinoceros diversity is threatened than of beetle diversity. Finally but importantly, such datasets can be used to check or validate habitat-based models and conclusions.

There are two databases on the status of animals in the wild that are relevant, that of the IUCN/SSC/Species Conservation Monitoring Unit (SCMU), and, for U.S. species, that of the U.S. Endangered Species Office.

The SCMU database is maintained by the SCMU (and International Council on Bird Preservation - ICBP) offices in Cambridge, England, on minicomputer facilities located at Kew Gardens. It is the only such database which attempts global coverage of wild species in danger, and additionally is linked with other sorts of relevant conservation data - i.e. the habitat dataset of PADU - the Protected Areas Data Unit of IUCN.

The SCMU database is first of all restricted to those species which have been taxonomically described, and which are thought for some reason to be potentially or actually in trouble. These restrictions lead to lower information quality and lower information quantity as one proceeds from terrestrial "higher" vertebrates (most mammals and birds) "down" to reptiles and amphibians, fish, and invertebrates.

SCMU assembles information and publishes reviews (Red Data Books) with revisions on a roughly 10-year cycle by Class and area - i.e. New World Mammals, Birds of Africa, etc. The data comes from informants identified internationally from research papers, and/or extensive personal contacts built up by each of the Red Data Book compilers. For example, the Mammal data compiler has had about 5,000 correspondents (informants and contacts) around the world.

At the time of publication, the information is generally the best available from such serendipitous sources. It includes apparent species range, estimates of remaining numbers of individuals, apparent threats, trends, etc. Species are classified into Endangered, Threatened, Vulnerable, Rare, or Indeterminate - the latter typically in the case where none have been seen in many years - but which often includes cases where no one has looked.

The main weaknesses in the system come from the chancy sources and from the long time interval between revisions. There are effectively no resources for survey and census. Even if a 10-year cycle of republication can be maintained for each group and area, 10 years is a long time. Crises can develop and pass beyond possible action in that interval.

The basic operating budget for this facility is below $325,000 per year - and plus a roughly like amount in contract income for specific surveys hired by outside organizations (Barzdo, 1985). This is a trivial budget against the center's responsibility of providing the major sourcebooks of the world's threatened species (Red Data Books). Presently, 40% of the base budget ($125,000) is a grant from World Wildlife Fund - U.S. (WWF, 1985).

Recent connection to computer services at Kew Gardens has permitted Red Data Book publications to track events somewhat more closely. Nonetheless the quite limited staff resources are not utilized as efficiently as they could be; small amounts of funding (e.g. $15,000) which would buy several microcomputers would greatly facilitate data compiling and publication (Thornback, 1985). Larger amounts of funding would enable both more timely reporting and at least catalyzing funds for survey and census.

On a negative note, the IUCN/SSC/SCMU has not had the resouces for field census, much less action. There are few other entities which have taken responsibility for any aspect of species conservation on a global scale. As a result, the timeliness of Red Data Book information may be of less consequence than otherwise. In real-world terms, rather few decisions and even less action hinges on changes in Red Data Book classification of species. There are not many organizations whose behavior depends on such data.

U.S. Office of Endangered Species Database

Somewhat analagous to the IUCN/SSC/SCMU database is that maintained by the U.S. Endangered Species Office. For species which occur or did occur within the 50 states, this system provides a source of information.

Given the more restricted range of the effort, more detail is attempted - for example, range information is down to county level, whereas SCMU's is down to state, province, or equivalent. The system is presently being computerized, with a trial database of 25 species operating in test mode at the time of this review (Gill, 1985). Opportunity to record extensive textual information and discrete data is provided in this system, but it is premature to tell how useful the retrieval services will be.

At the present time, only 350 species are listed (meaning believed to be Endangered or Threatened) by the U.S. Office of Endangered Species. A further 3,000 are held on an action listing for further investigation. The annual turnover rate for these 3,000 is about 10% - either taken off of the consideration list, or proposed for official designation as Endangered or Threatened. Thus the effective average time for decision may be of the same order as SCMU's planned Red Data Book revision time - about a decade.

When and if the Endangered Species Office decides a species' status warrants official listing, it must be approved at several levels of the U.S. Dept. of Interior, public comments solicited and reviewed, and finally approved or not approved by an Assistant Secretary of Interior. This process can be quite lengthy, and could lead to harmful delay in recognition of a problem. It also may create bias in the data available from this source. The rate of new listings varies considerably, for non-biological reasons.

The U.S. Endangered Species database could be improved by 1) speeding up the time for consideration of candidate species for listing, and 2) finishing the computerization of the data. Speeding up the consideration process would presumably require devoting more staff time to evaluation of the status and threats to species. This speedup is needed because the current mean time of 10 years for evaluation is far longer than the rate of change of species' threats. Finishing the computerized database would be a considerable help because (if it's successful) the resulting database could play a key role in accurately coordinating the activities of the many Federal agencies which have management control over wild lands.

ISIS - Captive Animal Database

The International Species Inventory System (ISIS) was created in 1974 in recognition of the fact that the last chance for a growing number of wild animal species was a captive population. Such populations are the only possible action when the habitat of the wild species will temporarily, at least, be untenable. Captive populations can also offer very desirable insurance when a species is at risk of loss in the wild. An increasing number of wild species, especially the large and easily hunted ones, will depend on captive populations to survive through periods of civil unrest, habitat catastrophe or mismanagement. There is emerging data to support the notion that such captive populations can be used to successfully restock empty habitat after the factor making the habitat untenable has been changed (e.g. Stanley-Price, 1984).

In the case of large edible or marketable animal species, poaching is a major global threat. The species' survival is threatened even though a habitat survey might show apparently tenable habitat. An accurate census by SCMU would only document the decline. In the growing number of such cases, a genetically and demographically viable captive population is the most certain method of retaining the species on the planet.

ISIS collects the detailed specimen information necessary for sound conservation-oriented captive-population management from participating zoological gardens, primate centers, and the like. Coverage has grown from 55 facilities when the system began in 1974, to 211 as of late 1985 (an expansion rate of one new facility per month. About 65,000 living specimens of 2300 species are included The system is not restricted to today's endangered species, as many more will be endangered in the future.

Information presently comes from facilities in 14 countries, but coverage is best for U.S. and Canadian institutions. The system is based at the Minnesota Zoological Garden, Apple Valley, Minnesota.

Information on captive populations has been collected using the simple method of a standardized specimen record. Over 750,000 of these have been provided to participating institutions thus far (they return one copy). Presently, a micro-computer software package is being tested for use by individual participants. The software automates their records, and ISIS reporting as well.

ISIS now publishes survey reports at six month intervals. They are current to within about 6 weeks at the time of publication. These show holding institutions, sex ratio, age distribution, fraction captive-bred, birth, death, and import trends for all species of mammals and birds held in captivity by participant facilities. Expansion to reptiles and amphibians is occurring with the microcomputer software step mentioned above. Detailed information on pedigree and data for demographic analysis is available by special order for any species.

ISIS' database and reports are consulted by the Office of Scientific Authority (CITES Advisory), Wildlife Permit Office, and Endangered Species Office, for several purposes.

ISIS is funded by participant zoological institutions, grants, donations, etc. Half of its annual budget has always been grants of 12 month duration. ISIS' total annual budget is $250,000.

The quality of ISIS data could best be improved by 1) improving international coverage, and 2) including information from commercial animal dealers and brokers. As captive animals move extensively for genetic management and other purposes, better international tracking would help considerably. This job could be accomplished with additional funds to deal with translation, currency exchange problems, etc.

The commercial animal dealers are very often a "black box" where animals lose their individual identity between the zoological seller and eventual zoological buyer. Though animals increasingly move on a loan or donation basis directly between zoos, the dealers still present a data obstacle for an important proportion of transfers. Effective encouragement of records-keeping and sharing by these enterprises will be required to solve this problem.

Future data gathering activities by ISIS are being made much more convenient and cost-effective for individual facilities and for ISIS by the development of microcomputer software for the purpose. This will make recording such data very easy for each facility, seems likely to expand coverage, and will automate pooling the information through ISIS. The chief need in this area is for funding to permit building regional computer "nodes" for continental regions often isolated from the rest of the world by quarantine, political, or economic barriers. Such areas include the United Kingdom, Australia, and possibly others. Such regions have special needs for regional information. Development of regional-level databases which communicate with regional zoos and also share and exchange information with the comprehensive ISIS database would improve data quality, and information services.

PROBLEMS AND SOLUTIONS

The basic problems are two:

1. How to better assess the situation and its risks.
2. How to reduce further damage.

Better assessment would require both effective habitat monitoring and expansion of the existing species by species monitoring programs.

Habitat monitoring programs, along with other scientific conservation activities, deserve increased governmental grant support. The biosphere is our life-support system. It supports our country, and our species. Failure to accurately monitor its health is far more risky than failure to monitor individual human health. Strategy and priority decisions have already been established by expert panels (NRC 1978, NRC 1980) - these need only updating to deal with the further losses in the intervening 5 and 7 years.

The international wild species monitoring effort of the CMC also badly needs additional support. A stable basic operating budget is the first need, followed by at least modest resources for catalyzing surveys.

A further need is for improved database sophistication at CMC, especially with regard to handling dynamic political boundaries in species range data, and for simple kinds of ecological parameters associated with species. A request of mine for lists of endangered species whose range was insular, was beyond the database capacity (MacKinder, 1984).

The more specialized databases, for U.S. species (U.S. Endangered Species Office), and for captive populations (ISIS), have special needs. The Endangered Species database needs to reduce the time required for listing decisions, and the forthcoming computerized database will need review of content and performance. With the growing importance of captive populations for preservation of diversity, ISIS needs a stable basic operations budget.

Given that the world's ecosystems are being made into separated fragments in reserves, etc., it is vital that the consequences of fragmentation be carefully studied, and that effects of different patterns be understood. Only one such study (Lovejoy, et. al., 1983) is being performed before the fact - with assessment prior to the fragmentation. At least a single parallel study in each of the other major habitat types is essential; to make it science, there should be two independent studies in each.

The U.S. cannot be self-sufficient regarding the biosphere - vital national interests are affected by ecological actions of others outside the U.S. borders. In this situation, it is past time for recognition of conservation as an area critically needing scientific specialists, and needing development of technology. Conservation biology must become a legitimate scientific discipline, with all that implies in terms of institutions, professional training, and grant support.

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Contract Paper No. 2

Status and Trends of Domesticated Animals

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Winrock International

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BIOLOGICAL DIVERSITY:
STATUS AND TRENDS FOR AGRICULTURAL DOMESTICATED ANIMALS

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Animals are domesticated by people to serve the needs and wishes of people (table 1). Biological diversity between and within animal populations provides the raw material from which domesticated animals can be molded to better serve these needs. Therefore, loss of biological diversity is akin to the erosion of an essential natural resource base. In the current presence of plenty, the loss may go unnoticed. However, the future holds unknown, but surely greater needs. Declining biological diversity may mean, at best, lost opportunities for improving animal productivity and, at worst, an inability to meet future human needs for animal products.

Biological diversity results from the combined, often interacting, effects of genotype and environment. Much of the art and science of animal husbandry is devoted to manipulating the environment to improve animal productivity. Generally, the extent of environmental manipulation is directly determined by short-term costs and benefits. This report, however, is primarily concerned with the current status and trends of longer term genetic influences on biological diversity within and among populations of agricultural domesticated animals.

GENETIC DIVERSITY

Genetic diversity within the animal kingdom has resulted from eons of accumulated mutations (small changes in the genetic messages transmitted between generations). Generally, mutations yield a nonsense message, just as would an arbitrary change in the spelling of a word. Occasionally, however, a new operable message results. When this new message improves fitness, individuals carrying the favorable message produce more progeny, thereby increasing frequency of the favorable genes in the population.

Selection, migration, and chance all likely played a role in altering genetic diversity within the foundation populations of domesticated animals. The initial domesticates were quite possibly "selected" for being a bit more tractable (or, perhaps, just slower of foot). Once domesticated they became genetically isolated from the parent wild population (except the occasional mating of domesticated females by wild males). This isolation provided opportunity for changes in gene frequency through chance or, more importantly, through selection for favorable genes or against unfavorable genes. Because the interbreeding sample would be relatively small, inbreeding would tend to expose deleterious alleles, causing them to be removed from the population, thereby decreasing the "genetic load" carried by the domesticated sample compared to that of the wild population.

Selection is the process by which individuals carrying favorable genes leave more progeny than do inferior stock. In time, the superior genotypes will prevail throughout the population. Nature favors those genes that improve fitness to survive and thrive in the natural environment. This we now understand as the mechanism of natural selection, described by Darwin as "survival of the fittest."

Man may impose a different definition of superiority. His preference may be for high levels of milk production, lean meat, or fine fibers. Often these human preferences reduce fitness. Housing, nutrient supplements, and predator protection may be required for highly selected, highly productive animals no longer able to fend for themselves in the natural environment.

Genetic diversity is maintained and augmented by sexual isolation. Matings between species are rare. Even when matings occur, progeny from interspecific matings are usually infertile.

Within species, members of different genetic groups (such as races and varieties) are interfertile. However, genetic diversity between groups has arisen over time because members of one group do not freely and randomly mate with members of other groups. For example, sheer distance contributed to the development of the distinctly different cattle subspecies -- the European *Bos taurus* and the Asian *Bos indicus*.

For species of farm livestock, the term "breeds is commonly used to designate interfertile subpopulations of the species. As defined by Lerner and Donald (1966), a breed exists..." for practical purposes, a) when it has some identifying features, or b) when it has a formal association of breeders, or c) when certain government officials say it is (a breed)."

Identifying features that are used to distinguish breeds include color, body size and shape, type and fineness of coat fibers, level of milk yield, and degree of adaptation to disease and climatic stress. Establishment of breeds by formal association or government edict is a practice largely confined to developed regions where rules and regulations effectively limit intermating between members of different breeds. In developing regions, limitations on intermating between breeds tend to be more a consequence of geographic isolation.

Genetic diversity between "breeds" primarily arises from differential intensities and directions of selection, both natural and artificial. One or more livestock breeders in a region may establish particular selection goals and, then, through artificial selection develop breeds or lines that meet these goals. Selection is generally combined with controlled matings so that members of the line or breed become more closely related than average for the species. This "inbreeding" tends to fix type and increase the similarity between parent and progeny and increase diversity among lines. Often, the sexual isolation among different breeds is maintained by the formation of pedigree associations that only register progeny from purebred matings. Males not considered suitable to sire offspring may be effectively removed from the breeding population by castration.

The processes for manipulating genetic diversity in domestic populations are treated in more detail by other authors in this series (e.g., Notter, 1985).

DOMESTICATION

Domestication implies that man controls reproduction of individuals and(or) groups, essentially determining which males and females contribute offspring to the next generation. Generally, man also assumes some responsibility for managing nutrition and health of domesticated animals.

Domesticated animals share two basic characteristics:

- domesticability
- purposes or products desired by people

The first is an essential characteristic. Individuals may be tamed, but relatively few species have proven readily domesticable. The second characteristic largely determines the time and effort that people are willing to invest in the domestication process. For example, candidates for domestication still exist. However, little effort will be expended in their domestication, as long as there are domesticates that already fit the production/market environment -- even if the candidate potentially fits better than the domesticate.

The first species domesticated about 12,000 years ago are thought to have been the dog and, somewhat later, the pig (Bokonyi, 1983). Both species readily adapted to feeding on the campsite wastes of hunter/ gatherers and served as living meat stores for periods of scarcity. Subsequently, herbivores (including ruminants, cameloids, and equines) were favored for domestication because they converted grass, which man did not eat, into living stores of meat, milk and blood.

When man ventured into inhospitable habitats -- such as deserts, high mountains, and frozen tundra -- adapted species such as camels, yaks, llamas, and reindeer became prime candidates for domestication.

Although domesticated animals probably first served as living meat stores, additional products and purposes would soon become apparent. Females could be milked to provide a daily nutrient supplement. Dogs joined in the hunt, helping to control the flight of game (not unlike their future role in herding livestock). Loads carried on the backs or pulled on sledges behind animals were lifted from human shoulders. Eventually, animals were carrying people as well as their packs and pulling plows as well as sledges. The variety of ways in which domesticated animals serve people has continued to expand (table 1).

Practically all the domesticated animals are vertebrates from the phylum Chordata (figure 1). Mammals and to lesser extent birds are predominant (figure 2). Major exceptions are the honey bee and silkworm and, recently, crustacea and mollusks, which are being "farmed." The controlled breeding and management inherent in domestication have also been applied recently to commercial production of several fish species, including salmon, trout, catfish, and tilapia.

CANDIDATES FOR DOMESTICATION

Principal candidates for domestication are generally similar to already-domesticated types; i.e., uses or markets for their products or purposes are already established. Often these candidates are already well adapted to an environmental niche not filled by an important domesticate. Examples are the capybara in the wet lowlands of South America (Gonzalez-Jimenez, 1977); the manatee and dugong in subtropical waterways; the seal and walrus in cold temperate waterways; several types of pigs, cattle, and buffalo indigenous to tropical Asia (NRC, 1983); and game species such as the eland and oryx grazing semiarid African rangelands (Thresher, 1980).

Particular attention has been paid to the economic potential of several species in the family Cervidae (table 2). Farming of red deer in New Zealand has become a major commercial industry annually exporting millions of dollars of vension, hides, antler velvet, and slaughter by-products (Drew and McDonald, 1976).

GENETIC DIVERSITY BETWEEN AND WITHIN DOMESTIC POPULATIONS

Much of the more useful biological diversity resulted from the evolution of special adaptations before animals were domesticated. Examples include the major differences between species in water requirements (King, 1983) and the structural differences among the gastrointestinal tracts of herbivores (Demment and Van Soest, 1983).

Ability to regulate water turnover is a major adaptive mechanism for livestock grazing dry rangelands, especially where drinking-water sources are not well developed. The oryx and camel are well suited to these production systems (table 3).

The ratio of metabolic rate (MR) to gut capacity (GC) has been proposed as a principal factor influencing evolution of the gut form and feeding strategies of herbivores (Demment and Van Soest, 1983). Because MR is proportional to metabolic weight ($W^{.75}$) but GC is proportional to weight (W), the ratio (MR/GC) decreases with increasing body weight.

Strategies by which herbivores consume and digest sufficient nutrients per day differ depending on body size. Small herbivores (rodents, rabbits) have evolved hindgut fermentation and selectively feed on highly digestible low-fiber foods; caprophagy is a common means of recovering nutrients (especially vitamins and proteins generated by gut microbes) lost through rapid passage. Medium-sized herbivores, primarily ruminants, can consume a higher-fiber ration because their reduced MR/GC ratio allows delayed passage and longer digestion time. The complex structure of the multiple-compartment ruminant stomach increases fiber digestion efficiency by retaining large particles until they can be digested. Large herbivores such as elephants have such a relatively low MR/GC that they can depend on consuming sufficiently large quantities of food daily.

Man has made major changes in the diversity of livestock through directed selection. Examples include the extension in days lactating and milk yield per day of dairy cows. Cows lactating to meet the requirements for weaning a calf ordinarily produce 100 liters to 300 liters in 5 to 8 months; selected dairy cows on high-quality rations produce more than 20,000 liters in 10 months. Another dramatic example is the increased annual egg production from 30 to 300 for high-producing commercial poultry lines.

Measures of genetic variation within a population include the proportion of polymorphic loci and the average level of heterozygosity (Braend, 1981). Randomly sampled blood-protein loci are frequently evaluated because polymorphisms can be screened electrophoretically. Analyses of this type for wild animal species have commonly indicated percentage polymorphic loci in the range of 20% to 40% and average percentage heterozygosity of 5% to 15% (Nozawa, 1980). Based on limited analyses for samples from domesticated species (cattle, sheep, horses, and dogs), Nozawa reported genetic variation within these species similar to that reported for wild species. However, he found considerably less variability within samples from Japanese goat populations, a result he attributed to the limited sample of dairy goats imported from Europe to establish the Japanese dairy goat population.

Variation among 30 polymorphic loci was used by Kidd (1974) and coworkers as the basis for phylogenetic analysis of European cattle. Because these loci were assumed not to be subject to direct artificial selection, the "genetic distance" between breeds was postulated to be a result of differences in origin and period of reproductive isolation. Multivariate analysis of the data did in fact suggest distinct genetic differences and similarities among the 12 European breeds analyzed. Practical applications of such analyses could include setting priorities for preservation of threatened breeds, with priority given to threatened breeds genetically distant from nonthreatened breeds.

Less esoteric measures of variation between and among breeds are accomplished through contemporary performance comparisons of different breeds and crosses. In this way environmental influences on performance measured at different times in different places are largely removed so that differences between groups reflect genetic effects.

Increasing emphasis is being given to contemporary comparison of breeds. In 1970 USDA undertook a comprehensive evaluation of cattle breeds at the Roman L. Hruska U.S. Meat Animal Research Center in Nebraska. To date, over 20 breeds and several hundred breed combinations have been evaluated for growth, reproduction, milk yield, feed conversion, carcass, and other traits that influence efficiency of beef production efficiency (Cundiff et al., 1984).

Many of the same cattle breeds have also been evaluated in New Zealand. Baker (1983) compared results from U.S. and New Zealand trials for two composite traits (table 4). The first composite trait was average progeny carcass weight per cow calving, indicative of breed's value as the terminal sire line in crossbreeding schemes. The second was the weight of calf weaned per crossbred cow mated, indicative of breed's value as a sire line to produce breeding females. New Zealand comparisons of cow performance were conducted under two environments -- harsh and good. Results are ranked relative to the averages for Hereford-Angus crosses.

Results from these comparisons indicate substantial useful variation among breeds for traits important to beef production. Continental breeds such as Charolais and Chianina were originally selected for draft purposes; their large mature body size contributed to more rapid growth rates of slaughter progeny. Dairy breeds, such as Friesian and Jersey, contributed to increased weaning weight through increased milk yield of dam. Ranking of breeds between U.S. and New Zealand environments was generally similar; however, there were important differences in rankings for maternal and reproductive traits between the good and harsh environments in New Zealand (Baker, 1983).

LOSS OF GENETIC DIVERSITY

Extinction of domestic animal species is unlikely, if for no other reason than that domesticated animals are so closely associated with and protected by man. However, the loss of specific genes and genotypes is likely, especially when these are not currently the most economically competitive. A particular case in point is the substitution of "improved" breeds from developed countries for local, adapted types in developing countries.

Concern for conserving genes and genotypes stems from the knowledge that production and market conditions do change. Perhaps the lost genotype would be the one most suitable for a future environment or market. Recent successes in transferring genes between species and other forms of genetic engineering further argue for the conservation of rare and unusual genomes to ensure their availability for future use.

Land (1981) proposed a breeding strategy for promoting and conserving genetic variation between breeding lines for future application. This strategy involves deliberate selection to increase differences for production and fitness traits between lines. The hypothesis is that one or more of these lines would provide needed genetic material appropriate to future production/market conditions. Crosses among appropriate lines should also benefit significantly from both hybrid vigor and complementarity.

Loss of genetic diversity can result from fixation of alleles due to intensive selection, inbreeding, or both. However, analyses of domestic livestock populations show relatively little loss of genetic diversity due to these factors (Fredeen, 1984; Hudson and Van Vleck, 1984; Kennedy, 1984). Lack of continuity and consistency in breeding objectives and deliberate efforts by producers to avoid inbreeding may account for the relative lack of loss of genetic diversity in the past. However, the technologies of embryo splitting and transfer may allow for more intensive selection combined with reduction in the effective size of the breeding population; thereby, accelerating future losses of genetic diversity.

CURRENT STATUS AND TRENDS

None of the domesticated animal species appear in danger of extinction. However, potential for loss of genetic diversity within several species is a matter of concern, especially for a number of poorly characterized breeds and types in developing regions. A principal danger stems from replacement of these breeds by imported genotypes favored for the requirements of the current production/market environment.

Populations of several candidates for domestication are similarly threatened. For example, the wild progenitors of cattlelike species (genus *Bos)* such as the banteng, yak, and guar (or mithan) are now quite rare (Mason, 1980). Their habitat has been progressively reduced by human encroachment and competition from domesticated animals. Effective population sizes of the domesticated lines of these species are reduced when females are mated to cattle males. Crossbred male offspring are usually sterile.

Many of the breeds and types of domesticated species found in developing countries are in danger because of unrestricted crossing with improved breeds from developed zones. The threat of loss of potentially useful genetic variation has heightened in recent years for those species in which artificial insemination (AI) is widely used (especially cattle). As Osman (1983) pointed out, owners of a few cows often prefer using AI to incurring the expense of keeping a bull. However, few developing countries have the necessary facilities to collect and freeze semen from local adapted breeds. Therefore, AI is with semen imported from commercial studs in developed countries.

Crossbred progeny from these matings usually perform well, combining the advantages of additive genetic effects for improved milk yield or growth rate with hybrid vigor. Often the loss of adaptation to local environmental constraints (climate, disease) is masked by hybrid vigor in the first crosses. However, continued upgrading to the "improved" genotype without selection for fitness may lead to disastrous loss of ability to survive under local conditions without improved nutrition, health care, and other managerial interventions.

Cattle

Measured in terms of numbers and geographic distribution, cattle are the most successful of domesticated animals (table 5). Over the past decade, however, cattle numbers have actually decreased in North America, Europe, and Oceania, primarily because of declining markets for milk and beef and poor profitability. Two-thirds of the world's cattle are in developing regions; these populations continued to expand during the past decade.

Cattle are commonly classified in two subspecies, *Bos taurus* and *Bos indicus*. Breeds and types of the former are primarily found in temperate regions (generally the developed regions); those of the latter are primarily found in the tropical regions of Asia, Africa, and Latin America (table 6). Important exceptions exist to these general locations. For example, the American Brahman was synthesized from *Bos indicus* breeds imported into the United States earlier this century. The Brahman has had major impact on commercial beef production through crossbreeding with *Bos taurus* breeds such as the Hereford, Angus, and Charolais. New U.S. breeds formed from Brahman crosses, including the Santa Gertrudis (5/8 Shorthorn, 3/8 Brahman) and Brangus (5/8 Angus, 3/8 Brahman), combine the beef character of British breeds with adaptation of the Brahman. Another important exception is the N'dama, a *Bos taurus* type, found in western Africa. The N'dama has evolved resistance to trypanosomiasis, the tsetse-fly-carried disease that sharply limits productivity of domestic livestock throughout central Africa.

Most of the 270-plus recognized breeds of cattle originated in Europe and were then distributed through the world by colonialists. With major changes in production conditions and market preferences a number of these breeds have declined in popularity and may now be considered endangered (table 7). Fortunately, most of these breeds are relatively well characterized and steps are being taken to conserve potentially useful germplasm (Maijala et al., 1984).

There is reason for more concern for a lack of knowledge about a number of threatened breeds and types that are adapted to conditions of tropical developing countries. Examples of breeds at risk are the criollo of Latin America (De Alba and Kennedy, 1984; Wilkins, 1984), the Sahiwal (Hodges, 1984), and nine African breeds (table 8). The circumstances threatening survival of the African breeds are generally indicative of the problems, causes, and consequences for endangered cattle breeds in other parts of the world. For example, most are primarily used in traditional, low-input production systems by subsistence producers who cannot afford the high-cost management inputs required by breeds with higher production potential. Replacement of these adapted breeds with supposedly higher-potential imported breeds could actually lower output of meat and milk.

Buffalo

This species is generally subdivided into two types: the swamp type of southeastern Asia and the riverine type of the Indian subcontinent (Cockrill, 1974). The river type is also found in Egypt and Mediterranean Europe (mozarella cheese is made in Italy from buffalo milk). The river types tend to be larger; several breeds have been selected for milk production. Both types are used for draft. The swamp buffalo is the power source for cultivation in the rice-paddy systems of China and southeastern Asia. Buffalo imported from Asia to Trinidad and Brazil are now primarily used for meat production; in Trinidad, they are known as "beefalypso" (Rastogi et al., 1978).

Relatively little effort has been directed to improving the productivity of buffalo, especially the swamp type (Chantalakhana, 1984). This benign neglect of an important species has been noted by many who have urged establishment of genetic-improvement schemes. On the positive side, however, there has been no apparent loss of genetic diversity for fitness traits due to misdirected efforts to improve milk and meat yields.

Sheep

Indicative of the genetic diversity among sheep, over 300 breeds are identified by Mason (1969). These breeds include a number selected for milk, meat, and, especially, wool production; however, the majority serve multiple purposes (table 6).

Sheep are often classified according to coat and tail type (Foote, 1978). Three types of fiber are found in sheep coats: kemp, hair, and wool. Kemp is a medullated (hollow), coarse fiber similar to the hairy outer coat of wild sheep. Hair is the finer, medullated fiber on the head and legs of many wool sheep, although hair covers the body of tropically adapted hair sheep (Fitzhugh and Bradford, 1983). Wool is the commercially valuable fiber; it is finer than hair, generally solid. Scales on the wool fiber facilitate spinning and felting. Grades of wool range from fine (15 µm in diameter), which is used in clothing manufacture, to coarse (40 µm), which is used for making carpets.

Tail length and type varies considerably among breeds. Compared to wild sheep, which have 13 or less coccygeal vertebrae, domestic sheep vary from 10 to 35 vertebrae. Some breeds, such as the Awassi, a Middle Eastern dairy/carpet-wool type, deposit such heavy quantities of fat around their long tails that assistance during mating is required. Others, such as the Somali Blackhead, have short tails with large fat deposits on the rump. Most European breeds have long, thin tails.

The Finnish Landrace is an example of an important genotype that was almost lost (Maijala, 1974). Growth rate and wool quality are only mediocre. Competition from imported superior performance breeds caused numbers in Finland to decline sharply until animal breeders in other countries became interested in the high levels of reproductive efficiency exhibited by the breed. Breeding stock were imported into Great Britain and North America in the 1960s, where they have been incorporated into commercial maternal lines for mating with large meat-type breeds such as the Suffolk.

Goats

Goats are thought to have been the first ruminants domesticated. Mason (1969) identified 69 goat breeds, including specialized breeds for milk and fiber production. Among the latter are Angora (mohair) goats primarily found in Turkey, southern Africa, and Texas, and the Kashmiri (cashmere) from Tibet, China, and northern India.

The vast majority of goats are found in dryer developing regions (table 6), although a few breeds such as the Southeast Asian Kambing Katjang are found in the more humid tropics. The more important goats kept in the United States include the Angora and "Spanish" meat goats in the southwestern United States, and the dairy breeds (Saanen, Alpine, Toggenberg, Nubian, and La Mancha).

Genetic diversity between and within goat breeds does not appear threatened, probably because goats thrive over such a wide ecological range, primarily in developing countries. They tend to be survivors, among the last of the domesticated species that can live in drought-stricken or degraded rangelands (Winrock International, 1983).

Camilidae

The Camilidae family includes two genera: the Old World, *Camelus*, and the New World *Lama*. Both genera have adapted to unusual, relatively harsh environments: Asian and African deserts and the high Andean altiplano of South America, respectively.

There are two types of old world camels. The bactrian (two humped) was domesticated in southwestern Asia (Iran) and taken eastward as far as Mongolia and China. The dromedary (one-humped) was domesticated in southern Arabia and taken into the deserts of northern Africa and the Middle East. Various physiological adaptations enable camels to survive for weeks without drinking water (de Vos, 1982). This and the capability to produce milk and provide transport while browsing desert vegetation make the camel a particularly valuable animal to pastoralists living in the deserts of Asia and Africa.

Increasing recognition is being given to the need for better exploiting the camel. The International Livestock Centre for Africa (ILCA) has established a unit to compile and analyze information on the camel (Wilson, 1984).

The genus *Lama* includes two domesticated species, the llama and alpaca; one semidomesticated species, the vicuna; and one wild species, the guanaco. Llamas serve primarily as sources of meat and beasts of burden; alpacas are primarily noted for fine-quality hair and pelts. Alpaca and vicuna fibers commonly sell on the international market for twice or more the price of fine wool.

Llamas and alpacas are productive at altitudes of 4,000 meters to which cattle and sheep are poorly adapted (Novoa, 1981). Low conception rates and high neonatal mortality are common, sharply limiting commercial offtake from these species and opportunities for genetic improvement.

Numbers of all four species have declined sharply since the Spanish conquest of the Incan empire. Loss of biological diversity has not been measured but has almost certainly occurred. Most seriously affected were the vicunas, which were thought to number only 12,000 individuals by the end of the 1950s. Slaughter for hair and pelts was the reason for decline. Fortunately, concerted efforts to control slaughter by the Government of Peru and a number of international agencies reversed the decline. By the end of the 1970s, the Peru population of vicuna was estimated to be 65,000 (Novoa, 1980).

Swine

Swine breeding in developed countries has followed the lead of poultry with intensive selection for performance (meat yield, growth rate, pigs weaned per litter) and combination of lines for hybrid vigor. Mason (1969) classified swine breeds as meat, lard, and bacon types. The lean meat type is most favored today in North America. Many commercial lines are synthetics formed from proprietary combinations of lines held by primary breeding firms. The carcass composition of breeds popular 40 years ago, such as the Hampshire, Poland China, and Duroc, has been radically changed in favor of higher lean yields instead of lard.

Considerable interest is currently expressed in sampling Chinese breeds, especially the highly prolific types that give birth to 15 or more pigs. Many Chinese types are also known for their capacity to utilize relatively high roughage rations in contrast to the grain-oilseed rations fed commercial pigs in the United States.

In much of the developing world, pigs scavenge household wastes. Inputs are minimal but productivity is low as well. These are literally the "piggy banks" of many small farmers. These local breeds, such as the criollo pigs of Latin America, are relatively resistant to many diseases and parasites. Unfortunately, this does not include resistance to the highly virulent African Swine Fever that was inadvertently introduced into Cuba, Brazil, and the Dominican Republic. Rather than risk the disease becoming established in this hemisphere, drastic measures were taken. All pigs, including the criollo pigs kept by most small farmers, on the island of Hispaniola (Dominican Republic, Haiti) were slaughtered; farmers' stocks are now being replaced with commercial lines from the United States. It remains to be seen how these introductions will fare under local farm conditions.

Poultry

Poultry production, especially chickens, has been effectively industrialized in the past 40 years. Strains derived from the White Leghorn breed dominate the egg industry; similarly, most broiler lines are derived from the Plymouth Rock and Cornish breeds. Most commercial turkeys are white, broad-breasted strains; most commercial ducks are derived from the white Pekin (Crawford, 1981).

Four classifications for poultry populations were defined during the Fourth FAO Consultation on Animal Genetic Resources (Crawford, 1984):

- industrial -- breeds for specialized egg or meat production under intensive commercial conditions
- middle-level -- breeds adapted to local, traditional farm conditions (generally referring to farms in developed countries); usually dual-purpose (eggs, meat)
- hobbyist -- fancy breeds kept because of unusual form, size, and feathers
- indigenous -- local breeds or types maintained as scavengers on farms (usually in developing countries); unselected for egg or meat production

The industrialized stocks produce the vast majority of poultry products consumed throughout the world. Breeding has been focused on increased productivity, depending on intensive management to resolve constraints on nutrition and health. Breeding of these commercial lines is dominated by a few firms (probably less than 20 worldwide). These firms generally retain a number of lines from which to make different crosses and selections. However, Crawford (1984) reported that the business planning horizon was only about a decade; it was not cost effective to retain stocks that might prove useful further in the future.

The middle-level stocks were appropriate to the good but relatively extensive management on North American family farms as late as the 1950s. Today, however, only a few hatcheries still produce these lines (whether chickens, turkeys, ducks, or geese). In Canada the number of such hatcheries had declined from about 800 to 13 in 1980 for all species. The typical barnyard fowl is vanishing rapidly because it is unable to compete with the high-yield, low-cost industrialized systems.

Hope that hobby flocks can serve as a reservoir of genetic variation seems unfounded. Crawford (1984) found that the fanciers tended to buy their seedstocks from the same few hatcheries. Most selection was devoted to picking the outstanding show bird, not necessarily the breeding lines.

The indigenous birds of most developing countries retain their behavioral and survival qualities. They scavenge household wastes, seeds, and insects. Eggs are often hidden from predators, including man. Semidomesticated strains such as mallard ducks and Canadian geese are found in zoos and parks but have very limited influence on commercial poultry production.

The highly productive, industrial poultry strains have certainly lost biological diversity, especially for fitness under rustic conditions. To date however, this loss has not proven a constraint to their expansion. Industrial strains packaged with improved nutrition and health management are being profitably transferred into many developing regions.

It seems likely that industrialized poultry systems will increasingly fill the needs of urban markets. However, rural producers seeking poultry to scavenge low-value feedstuffs will require adapted, disease-resistant strains even at the cost of much lower levels of productivity.

GENE BANKS, DATA BANKS, AND DATABASES

Gene Banks

The many breeds, strains, and types of domestic animals around the world remain the principal banks for storing useful genetic variation. However, it must be recognized that these banks are not genetic conservatories isolated from the effects of environment and market. Changes do and will occur in response to these factors.

Efforts to conserve genetic variation generally involve one of two approaches:

- living banks
    - maintenance of small samples of endangered breeds or types in zoos, parks, or private herds
    - establishment of randomly intermating gene pools of several breeds or lines
- storage of gametes and(or) embryos from endangered breeds or types

Results from genetic and economic analyses (Smith, 1984a, 1984b) favor frozen storage of gametes and embryos as compared to maintenance of living banks. However, storage is effective only for those species in which techniques for freezing sperm (cattle, sheep, goats, pigs, rabbits, horses) or embryos (cattle, sheep, goats, rabbits, horses) are well established (Renard, 1984). Poultry are a notable example among major domestic species where techniques for frozen storage of eggs remain to be developed. Techniques for freezing gametes or embryos for all domestic species can likely be developed if sufficient resources are devoted to the task.

On the assumption that freezing and storage of gametes and(or) embryos will be technically feasible, the problem remains of identifying the appropriate candidates for conservation. The European Association for Animal Production (EAAP) established a standing committee to consider such issues (Maijala et al., 1984). Criteria for identifying endangered breeds are presented in table 9 for four species. These criteria are based on the effective size of the breed in question. A breed was considered endangered when numbers of reproductively active males declined below 20 and the number of active females declined below a point determined by expected litter size and the population trend (stable or declining). Small numbers of males and(or) females increases rate of inbreeding with higher probability of chance loss of genes and, usually, loss of fitness. Based on these criteria, the EAAP group identified 241 endangered breeds from among a total of 1,263 European breeds of domestic livestock (table 7).

Priority was given to candidates for conservation from among the group of endangered breeds based on two principal criteria (Simon, 1984):

- The population constituted an original, independent, closed breeding group that was not closely related to other nonendangered or already conserved groups.
- The population carried a specific genetic value (e.g., superiority for a production trait, a major gene, a character of potential importance, or expectation of substantial heterosis in crosses with other populations).

Databases and Data Banks

These terms refer to similar but not synonymous entities. Turton (1984) makes the distinction that data banks generally hold numerical data; whereas, databases hold a mix of numerical and bibliographic information. Increasingly, however, the term "database" is used for all forms of information storage.

Four types of databases are used to maintain information on biological diversity between and within animal populations (table 10).

Inventory. The inventory databases are based on actual counts or "desk" estimates on numbers and productivity. These databases are particularly useful for comparisons between different populations (e.g., table 5, which indicates numbers of domestic livestock per geographical location) and for tracking trends in population numbers and productivity over time. Accuracy and precision vary considerably among inventory databases. In the United States, cattle and swine inventories are estimated several times annually by USDA. Reports of changes in numbers and weight can have a major effect on value of both animals and future contracts. By contrast, counts and productivity statistics for livestock populations in many developing countries represent more or less informed "guesstimates."

Pedigree. Maintenance of records of ancestry (pedigrees) is a principal function of most breed societies. Accurate pedigrees are particularly important to mating strategies involving inbreeding or outbreeding and to selection incorporating information on relatives (e.g., selection of dairy sires using milk yields of female relatives).

Performance. Performance databases, often in combination with records of ancestry have contributed to increasingly effective efforts to improve the productivity of domestic livestock. The Dairy Herd Improvement Association (DHIA) database currently collects and provides computerized performance information on 4.6 million cows (about 43% of the U.S. dairy herd). Accumulated computer files contain 30 million lactation records on over 14 million different cows. This database is used by dairymen to improve their breeding and management, by AI studs to select superior dairy sires, and by scientists to support research on the source and manipulation of biological diversity.

Performance databases are maintained for most domestic livestock species in the United States. Often these are relevant primarily to an individual herd, whether kept by a private breeder or a public research institution. Corporate breeding is especially important in swine and chicken industries. With the emphasis on profitability in these corporate operations has come increased emphasis on collecting, analyzing, and utilizing performance records.

Databases combining performance records with ancestry records opened the door to understanding the extent and sources of genetic diversity. The first estimates of heritability for commercial livestock were not made until the late 1930s, about the same time that hybrid vigor was first being utilized in poultry breeding in an organized manner.

Today, analysis of databases on livestock from both research herds and private farms has led to confident recommendations on manipulation of genetic diversity. For example, rules of thumb have been published and generally accepted for the relative degree and source of genetic variation between and within beef cattle (table 11). Similar rules of thumb are available for dairy cattle, swine, and poultry (although details about poultry are often closely held proprietary information), and to a lesser extent for sheep, goats, and horses.

This level of understanding about genetic diversity of livestock populations would not be possible without the rapid expansion of performance-pedigree databases for these species. The detailed information needed to estimate genetic parameters, such as heritability, genetic correlations, and heterosis, was generally first available for publicly supported research herds. Subsequently, however, livestock producers began to appreciate the benefits of keeping performance records to improve breeding and management decisions. Information available in U.S. beef cattle databases (tables 12, 13) is indicative of the type and extent of information now being collected by both private and public sectors (Winrock International, 1985).

Genetic statistics calculated with data from private herds provided the needed confidence that these values were appropriate to commercial management conditions. With this confidence has come acceptance and wide application of rules of thumb, such as those in table 11.

Such detailed performance databases are not yet available on most livestock populations outside the developed countries. Because production conditions differ, it is likely that rules of thumb based on analyses from developed countries are not entirely appropriate to livestock in developing countries. That is, they will not apply to the vast majority of the world's livestock for which improvements are needed.

Fortunately, efforts are underway at the national and regional level to develop these much-needed databases (FAO, 1984). Unfortunately, financial support for these efforts is limited.

Bibliography. The CAB bibliography database maintained by the Commonwealth Bureau of Animal Breeding, Edinburgh, Scotland, evolved from the Animal Breeding Abstracts published by the bureau since 1929 (Turton, 1984). The CAB database contains over 75,000 abstracts of publications on livestock reproduction and genetics. Species include all common farm livestock, poultry, elephants, farmed game, cameloids, fur bearers, dogs, cats, laboratory animals, and other economically important mammals. Particular attention is given to breeds and their performance. Coverage of worldwide literature is attempted; however, many of the irregular reports from developing countries are missed.

Winrock International with funding from USAID has developed bibliographic databases on several species of farm livestock. Orientation is to livestock in developing countries. Each entry includes title of report, author(s), source of report (journal, organization), country, and key word discriptors of the type of information reported. Currently the number of entries per database are goats, 7002; cattle, 1777; sheep, 1969; swine, 345; water buffalo, 1,753; poultry, rabbits, bees and other livestock, 1,258; pasture and livestock feeds, 448. A copy of the paper or report cited is on file for more than 11,000 of the 14,552 entries.

Efforts to better cover the literature on types and performance of livestock in developing countries have led to development of bibliographic databases for Africa by ILCA (Trail, 1984), for Asia by the Society for the Advancement of Breeding Researchers in Asia and Oceania -- SABRAO (Bhat, 1984), and for Latin America by the Asociacion Latinoamericana de Produccion Animal -- ALPA (Plasse, 1984). These efforts share in common an emphasis on collection and organization of data on performance of local breeds. The Food and Agriculture Organisation (FAO) has supported the development and maintenance of these databases (Rendel, 1984). Consultations have been held to promote:

- development of standardized definitions, nomenclature, and data-collection and -collation systems
- assistance to existing regional organizations and the development of new regional organizations for maintaining documentation systems
- development of two-stage data-bank systems:
    - initially emphasizing enumeration of breed populations, description of population structure, and collection of minimal data set of information on productive and adaptive characters,
    - followed in each country by more extensive documentation of performance and adaptive traits, and the environmental conditions under which performance was measured.

Constraints to Knowledge of Status and Trends

Data are the essential element for assessment of status and trends in genetic diversity. Databases on domestic livestock in most developed regions have improved tremendously in the last 20 to 30 years. Knowledge gained from analysis of data has led to significant advancements in livestock productivity and more effective business planning and operation by all elements of the livestock industry. The ultimate beneficiaries have been consumers, who have been provided better-quality animal products at reduced costs of production.

Developed Regions. Principal constraints to development of effective databases for all domesticated species in developed regions are economic. Those species or breeds that are not in the commercial mainstream or that do not have obvious future economic value receive little attention by either the private or public sector.

Organizations such as the Rare Breed Survival Trust in the United Kingdom and the International Union for Conservation of Nature and Natural Resources have made positive contributions both to documentation and conservation of breeds of lesser "importance." However, these organizations have sharply limited resources to apply to the task.

Too little is known about the sources of biological diversity. Data collected under contemporary environmental conditions are required to partition genetic and environmental sources of variation. Regulations prohibiting transfer of breeding stock and gametes between countries and regions can limit the development of useful comparative databases. These regulations are generally designed to protect both the health and markets of national livestock populations.

Quarantine facilities such as the Harry S. Truman center off the coast of Florida do provide a means of importing interesting, potentially useful genotypes into developed regions. However, quarantine can be a slow, expensive process.

Controlled transfer of gametes and(or) embryos provides another means by which different genotypes can be introduced for evaluation under common environmental conditions. Currently this approach is being evaluated by U.S. authorities as a means of sampling the diverse genotypes available in Asia (e.g., Indian cattle and Chinese swine) with little risk of introducing disease problems.

Developing Regions. Economic and institutional constraints to development and maintenance of comprehensive databases are even more limiting for livestock populations in developing countries. Experiment station herds are few in number; recordkeeping is often inconsistent. Private holdings of livestock are often quite small. Lack of individual identification, single sire herds, and(or) uncontrolled matings limit genetic analysis of performance data, even when collection of data on growth, milk yield, or other traits is feasible.

Regional efforts to survey livestock resources and compile bibliographic databases are being sponsored by SABRAO, the Interafrican Bureau for Animal Resources (IBAR), ILCA, and ALPA. FAO and other international organizations are supporting these efforts to identify and characterize the indigenous genetic resources.

As an example, the protocol for the ALPA database is indicated in figure 3. As described by Plasse (1984), first efforts have been directed toward:

- Development of computer programs
- Compilation, coding, and processing of all available data on Criollo cattle in Venezuela, Tabasco sheep in Mexico and a swine population in Mexico.

Future work will be directed toward extension of data banks on criollo cattle, sheep, and swine populations in Latin American and to establishment of databases on cameloids, goats, and poultry.

This work will include the following:

- Assemble existing literature on animal species of economic importance in Latin America, classifying it according to the traits included and their parameters, to environmental conditions and production systems, and to the scientific reliability of the source of information.

- Characterize animal species of economic importance in Latin America, with emphasis on those in danger of extinction, according to genotype, geographical distribution, objectives and type of production system, and priority for conservation. Provide quantitative information, derived from specific references, on important traits and their parameters together with information on the environmental conditions under which the data were obtained. Present a summary of the information included for each of the traits considered.

- Offer the user various options for the retrieval of the information, according to the classification criteria described.

The major costs of establishing these databases are the original collection and analysis of performance data. To the extent that results of these analyses are recorded in technical publications, annual reports and other media, the marginal cost for abstracting and storing data on computer files is relatively low.

These costs may include:

- Computer program development and computing costs
- Salary for technicians to abstract and enter data on computer
- Printing and mailing costs

Much of the effort of developing regional databases is volunteered by animal scientists in the region. The direct costs of mailings, computer charges, and administrative assistants are not great.

Plasse (personal communications) has estimated that approximately US$20,000 will be required in 1986 to continue establishment of ALPA database for cattle (table 14). Unfortunately, reductions in program budgets for 1986 may restrict financial support for these activities in Latin America.

The value of databases will be substantially diminished if they are not fully developed and maintained. Thus, consistent, continuing funding at modest levels is needed to ensure completion of efforts already started at regional levels.

International Board for Animal Genetic Resources

The recent CAST (1984) report, "Animal Germplasm Preservation and Utilization in Agriculture," calls attention to the need for an agency (International Board for Animal Genetic Resources - IBAGR) to undertake continuing responsibility for conservation of domesticated animal resources, establishment and maintenance of gene banks and databases. IBAGR would coordinate activities of national boards, which would concentrate on each country's particular genetic resources. Close coordination and collaboration with other regional and international agencies would ensure effective application of limited resources.

The proposed agency would be modelled after the already established International Board for Plant Genetic Resources. IBPGR is one of the international agricultural research centers whose support is coordinated by the Consultative Group for International Agricultural Research (CGIAR). IBPGR headquarters are in the FAO complex in Rome; related activities of the two organizations are generally closely coordinated.

FAO with UNEP funding also has an ongoing program in animal genetic conservation and management (Hodges, 1984b). This program includes a 36-member standing panel that evaluates needs and recommends strategies for such activities as development and maintenance of data banks, conservation of endangered genetic resources, and organization of training in conservation and management. A newsletter, Animal Genetics Resources Information, is published semiannually. Previously mentioned regional activities by SABRAO, IBAR, ILCA, and ALPA are part of the FAO supported cooperative efforts in animal genetic conservation.

Given the limitations on funding and other resources available, initiatives toward establishment of IBAGR should involve integration and continuation of current programs by FAO, and regional and national organizations.

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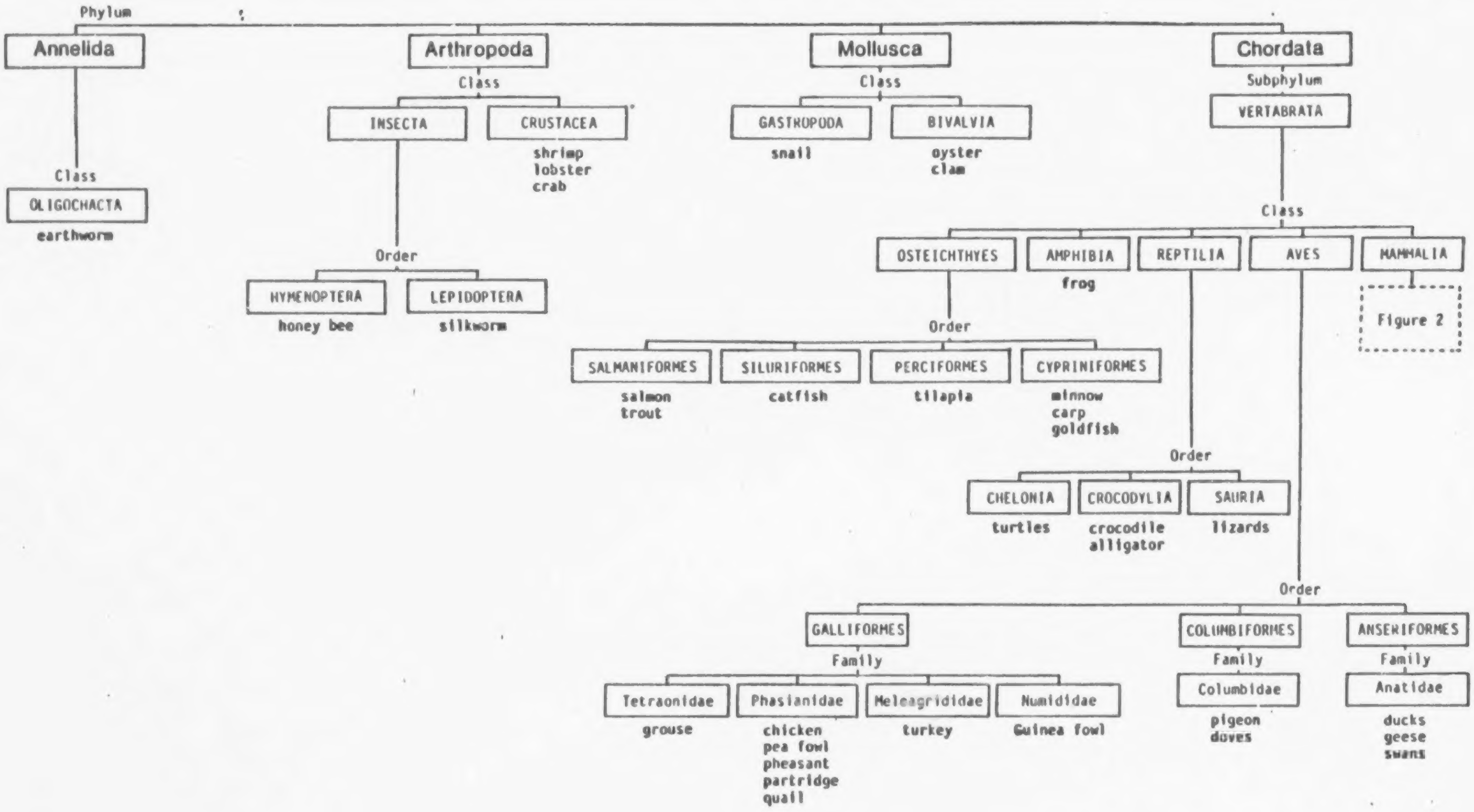
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Arthropoda
Mollusca
Chordata
Class
OLIGOCHACTA
earthworm
Class
INSECTA
CRUSTACEA
shrimp
lobster
crab
Order
HYMENOPTERA
honey bee
LEPIDOPTERA
silkworm
Class
GASTROPODA
snail
BIVALVIA
oyster
clam
Subphylum
VERTABRATA
Class
OSTEICHTHYES
AMPHIBIA
frog
REPTILIA
AVES
MAMMALIA
Figure 2
Order
SALMANIFORMES
salmon
trout
SILURIFORMES
catfish
PERCIFORMES
tilapia
CYPRINIFORMES
minnow
carp
goldfish
Order
CHELONIA
turtles
CROCODYLIA
crocodile
alligator
SAURIA
lizards
Order
GALLIFORMES
Family
Tetraonidae
grouse
Phasianidae
chicken
pea fowl
pheasant
partridge
quail
Meleagrididae
turkey
Numididae
Guinea fowl
COLUMBIFORMES
Family
Columbidae
pigeon
doves
ANSERIFORMES
Family
Anatidae
ducks
geese
swans

Figure 1. Place of domesticated agricultural animals in the Animal Kingdom.

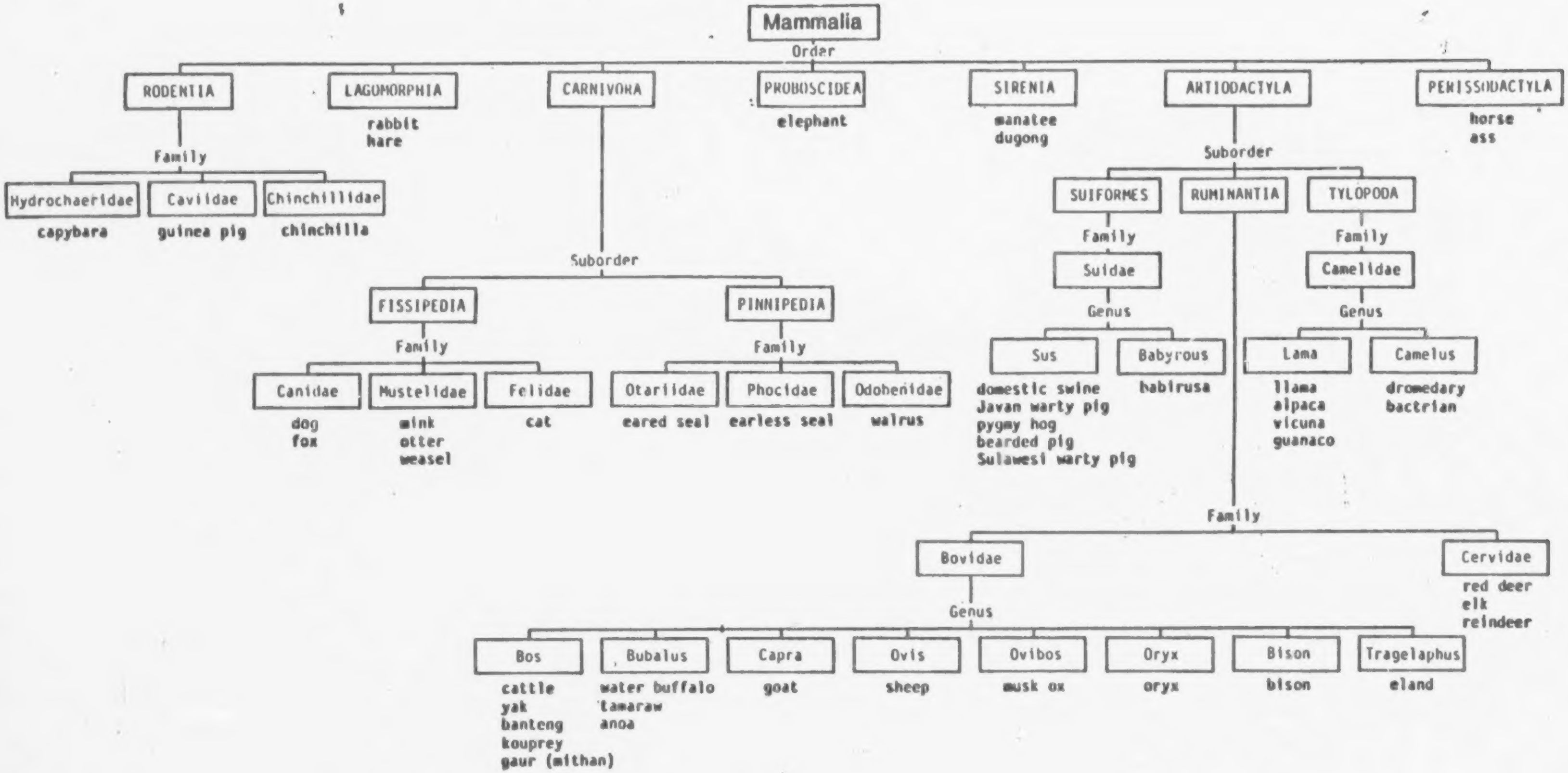
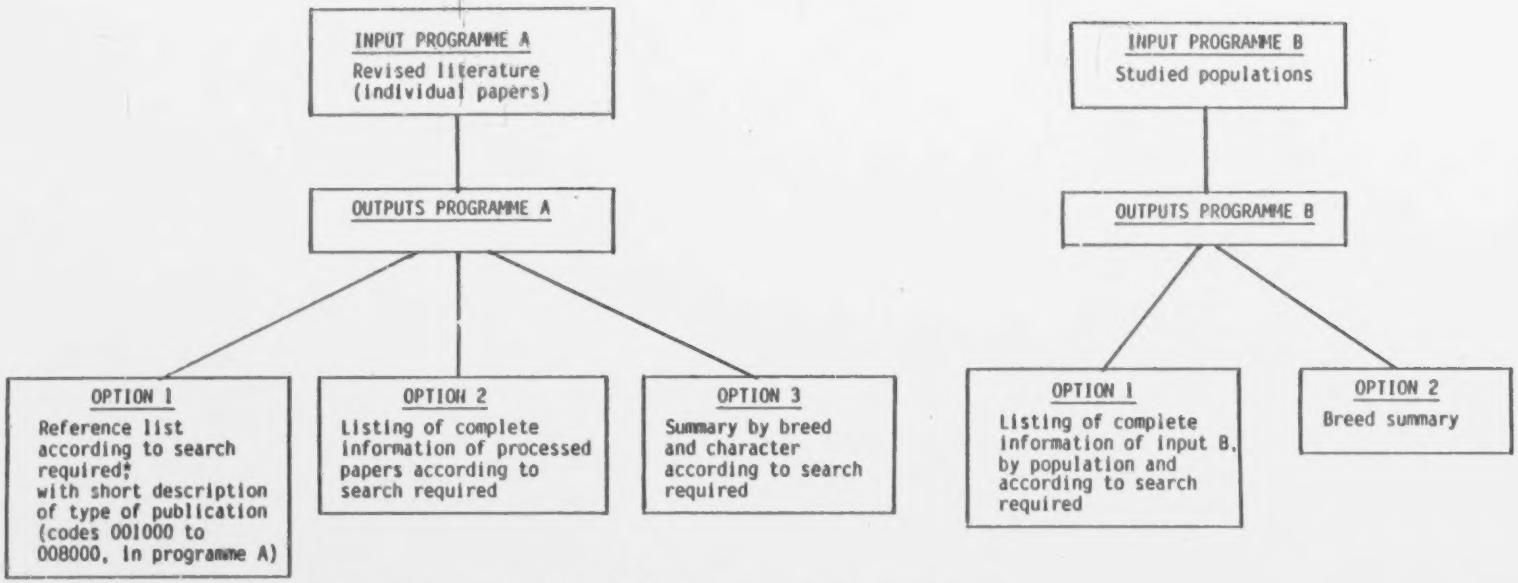

Mammalia
Order
RODENTIA
LAGOMORPHIA
CARNIVORA
PROBOSCIDEA
SIRENIA
ARTIODACTYLA
PERISSODACTYLA
Family
Hydrochaeridae
capybara
Caviidae
guinea pig
Chinchillidae
chinchilla
rabbit
hare
elephant
manatee
dugong
horse
ass
Suborder
FISSIPEDIA
Family
Canidae
dog
fox
Mustelidae
mink
otter
weasel
Felidae
cat
PINNIPEDIA
Family
Otariidae
eared seal
Phocidae
earless seal
Odobenidae
walrus
Suborder
SUIFORMES
Family
Suidae
Genus
Sus
domestic swine
Javan warty pig
pygmy hog
bearded pig
Sulawesi warty pig
Babyrous
babirusa
RUMINANTIA
TYLOPODA
Family
Camelidae
Genus
Lama
llama
alpaca
vicuna
guanaco
Camelus
dromedary
bactrian
Family
Bovidae
Genus
Bos
cattle
yak
banteng
kouprey
gaur (mithan)
Bubalus
water buffalo
tamaraw
anoa
Capra
goat
Ovis
sheep
Ovibos
musk ox
Oryx
oryx
Bison
bison
Tragelaphus
eland
Cervidae
red deer
elk
reindeer

Figure 2. Domesticated agricultural animals in order Mammalia.

Figure 3 ANIMAL GENETIC RESOURCES IN LATIN AMERICA
CATTLE DATA BANK
FLOW DIAGRAM


INPUT PROGRAMME A
Revised literature (individual papers)
OUTPUTS PROGRAMME A
OPTION 1
Reference list according to search required* with short description of type of publication (codes 001000 to 008000, in programme A)
OPTION 2
Listing of complete information of processed papers according to search required
OPTION 3
Summary by breed and character according to search required
INPUT PROGRAMME B
Studied populations
OUTPUTS PROGRAMME B
OPTION 1
Listing of complete information of input B, by population and according to search required
OPTION 2
Breed summary

*Search may be carried out for breed, environmental conditions, all productive characters, etc.

Source: D. Plasse (personal communication)

Table 1. Domesticated animals -- products and purposes.

Domesticated animals	Food Meat	 Milk	 Eggs	Fiber	Skins, fur, hides, leather	Transport, traction	Other
Ruminants							
Cattle	X	X			X	X	Manure (fuel, fertilizer), pharmaceuticals, horn
Sheep	X	X		X	X		Manure (fuel, fertilizer), pharmaceuticals, horn
Goats	X	X		X	X		Manure (fuel, fertilizer, pharmaceuticals, horn
Buffalo	X	X			X	X	Manure (fuel, fertilizer), pharmaceuticals, horn
Yak	X	X		X	X	X	Manure (fuel, fertilizer), pharmaceuticals, horn
Reindeer	X	X		X	X	X	Manure (fuel, fertilizer), pharmaceuticals, horn
Musk ox	X	X		X	X		
Cameloids							
Camels	X	X		X	X	X	Manure (fuel, fertilizer), pharmaceuticals
Llama	X			X	X	X	Manure (fuel, fertilizer), pharmaceuticals
Alpaca	X			X	X		Manure (fuel, fertilizer), pharmaceuticals
Vicuna				X	X		
Swine	X				X		Manure (fuel, fertilizer), pharmaceuticals, bristles
Equine							
Horse	X	X			X	X	Manure (fuel, fertilizer)
Ass	X	X			X	X	Manure (fuel, fertilizer)
Mule	X				X	X	Manure (fuel, fertilizer)
Elephant					X	X	Ivory
Poultry							
Chicken	X		X				Feathers, manure (fertilizer)
Turkey	X		X				Feathers, manure (fertilizer)
Ducks	X		X				Feathers, manure (fertilizer)
Geese	X		X				Feathers, manure (fertilizer)
Quail	X		X				Feathers, manure (fertilizer)
Guinea fowl	X		X				
Fish	X						Fertilizer, pharmaceuticals
Rabbit	X			X	X		
Guinea pig	X			X	X		
Carnivores							
Dog	X			X	X	X	Herding, hunting
Chinchilla					X		
Mink					X		

Sources: Turner (1971), McDowell (1977), Fitzhugh et al. (1978).

Table 2. Deer species recently domesticated or candidates for domestication.

Name	Origin	Adult male wt, kg	Gestation period, days	Principal products
Red deer *Cervus elaphus*	Europe, Near East[a]	95 to 300	233	Antler velvet, venison
Wapiti (elk) *Cervus canadensis*	North America, Asia	200 to 450	249 to 262	Antler velvet, venison
Sika (Japanese deer) *Cervus nippon*	Eastern Asia	45 to 80	222 to 246	Venison
Rusa *Cervus timorensis*	Indonesia	100	240	Venison
Caribou, reindeer *Rangifer tarandus*	Northern Europe, Asia, America	300	240	Draft, hides, venison
Musk deer *Moschus moschiferus*	Central Asia, China	9 to 12	178 to 192	Musk
Fallow deer *Dama dama*	Mediterrean Europe, Asia	50 to 80	226 to 230	Antler velvet

Source: de Vos (1982).

[a] Introduced to New Zealand, site of major farming activity.

Table 3. Comparison of daily water turnover and water consumed per kilogram of body weight.

Species	Water Turnover, ml	Consumption, ml[a]
Goat	94	65
Eland	83	68
Sheep	78	54
Zebu cattle	74	47
Camel	41	37
Oryx	35	29

Source: King (1983); refers to semiarid East African conditions.

[a] Does not include moisture in feed.

Table 4. Comparison of New Zealand and U.S. contemporary-beef-breed-evaluation results.

	Breed value as terminal sire: carcass wt per cow calving		Breed value as first-cross cows: weight of calf weaned weaned per cow mated		
			Environment		
			Harsh	Good	
Breed or cross	N.Z.	U.S.A.	N.Z.	N.Z.	U.S.A.
Angus purebred	88	93	80	96	NT
Hereford-X[a]	100	100	100	100	100
Friesian-X	107	NT[b]	125	129	NT
Jersey-X	92	88	110	113	104
South Devon-X	105	98	101	108	105
Charolais-X	101	98	88	98	102
Limousin-X	101	98	78	105	100
Blond d'Aquitaine-X	108	NT	81	108	NT
Simmental-X	104	98	87	121	109
Maine Anjou-X	105	103	99	109	112
Chianina-X	104	101	88	98	114

Adapted from Baker (1983).

[a] Hereford cross used as base for comparison, i.e., rank equals 100.
[b] NT = Not tested.

Table 5. Regional populations of major domesticated agricultural animals and % change since 1975.

Region	Cattle, 1000		Buffalo, 1000		Sheep, 1000		Goats, 1000		Camels, 1000	
	1983	% change	1983	% change	1983	% change	1983	% change	1983	% change
Developed										
N. America	126,817	-11	-	-	12,590	-17	1,447	2	-	-
W. Europe	100,912	-4	171	10	103,211	11	10,599	8	-	-
Oceania	30,564	-26	-	-	203,586	0	227	195	-	-
E. Europe & USSR	149,591	6	582	-17	181,537	3	8,169	8	240	-3
Other Developed	18,006	9	-	-	32,011	3	6,122	10	11	10
Subtotal	425,890	-4	753	-12	532,935	3	26,564	8	251	-3
Developing										
Africa	139,671	11	-	-	132,863	16	134,953	16	9,842	9
Latin America	269,917	4	578	113	113,091	3	32,370	6	-	-
Near East	56,798	21	3,663	-1	163,028	16	69,313	6	3,729	-6
Far East	268,542	7	97,524	9	73,554	14	132,150	17	1,950	5
Asian CPE	63,994	3	21,608	3	121,872	12	80,656	13	1,950	5
Other Developing	570	0	-	-	29	4	130	8	1,180	-
Subtotal	799,492	7	123,373	8	604,437	12	449,572	13	16,701	5
Total	1,225,382	3	124,126	8	1,137,372	8	476,136	13	16,952	5

Table 5. Continued

Region	Pigs, 1000		Horses, 1000		Mules, 1000		Asses, 1000	
	1983	% change	1983	% change	1983	% change	1983	% change
Developed								
N. America	63,857	5	10,580	19	5	0	4	0
W. Europe	113,690	17	2,451	-19	469	-37	769	-27
Oceania	2,923	7	532	-1	-	-	5	0
E. Europe & USSR	136,536	9	8,199	-18	52	-5	778	-14
Other Developed	11,818	30	257	-8	16	0	215	0
Subtotal	328,823	11	22,019	-3	542	-34	1,771	-19
Developing								
Africa	9,580	39	3,479	7	2,230	7	9,320	9
Latin America	78,590	10	21,147	-3	7,016	6	7,457	-5
Near East	278	21	1,698	-7	530	-1	8,682	0
Far East	34,811	25	2,560	0	212	10	3,644	17
Asian CPE	319,621	14	13,199	-2	4,465	42	9,002	9
Other Developing	1,895	20	89	31	-	-	-	-
Subtotal	444,776	14	42,172	-2	14,453	15	38,105	5
Total	773,599	13	64,191	-2	14,995	12	39,876	3

Table 5. Continued

Region	Chickens (millions)		Ducks (millions)		Turkeys (millions)	
	1983	% change	1983	% change	1983	% change
Developed						
N. America	455	-4	2	0	19	12
W. Europe	858	6	14	0	50	43
Oceania	52	4	-	-	1	0
E. Europe & USSR	1,423	36	11	-21	66	83
Other Developed	355	23	1	0	6	100
Subtotal	3,143	18	28	-10	142	54
Developing						
Africa	553	38	8	60	3	200
Latin America	1,023	45	14	40	16	23
Near East	314	41	4	33	4	33
Far East	809	39	73	24	-	-
Asian CPE	1,217	57	39	3	-	-
Other Developing	5	25	1	0	-	-
Subtotal	3,921	46	139	30	23	28
Total	7,064	32	167	21	165	50

Source: FAO Production Yearbook (1984).

Table 6. Numbers of breeds of domesticated livestock classified according to purpose and geographical origin.

Species/purposes		North America	Europe & USSR	Australia & New Zealand	Latin America	Asia	Africa	Total
Buffalo	Dairy		-			7	-	7
	Dairy, draft		-			1	1	2
	Dairy, draft, meat		1			-	-	1
Cattle								
Bos taurus								
	Dairy	2	42	1	2	1	2	50
	Meat	1	15	2	2	3	3	26
	Draft	-	8	-	-	-	3	11
	Dairy, meat	2	46	1	2	3	1	55
	Dairy, meat, draft	-	45	-	1	-	-	46
	Dairy, draft	-	3	-	-	1	1	5
	Meat, draft	-	19	-	-	1	1	21
Bos indicus (zebu)								
	Dairy	-	-		-	4		4
	Meat	1	-		1	-		2
	Draft	-	-		-	15		15
	Dairy, meat	-	1		-	1		2
	Dairy, meat, draft	-	-		-	3		3
	Dairy, draft	-	-		-	13		13
Zebu type								
	Dairy	1			1		-	2
	Meat	3			4		4	11
	Draft	-			-		4	4
	Dairy, meat	-			-		1	1
	Dairy, meat, draft	-			-		3	3
	Dairy, draft	-			-		3	3
	Meat, draft	-					1	1

Table 6. Continued

Species/purposes		North America	Europe & USSR	Australia & New Zealand	Latin America	Asia	Africa	Total
Goat	Dairy	1	25		-	5	-	31
	Meat	-	1		1	3	6	10
	Hair	-	1		-	2	1	4
	Dairy, meat	-	5		-	5	-	10
	Dairy, hair	-	1		-	2	-	3
	Meat, hair	-	-		-	5	-	5
	Dairy, meat, hair	-	-		-	6	-	6
Sheep	Dairy	-	1	-	-	-		1
	Meat	1	3	-	3	3	8	18
	Wool	3	20	4	-	6	2	35
	Dairy, meat	-	-	-	-	4	1	5
	Dairy, wool	-	8	-	1	8	1	17
	Dairy, pelt	-	5	-	-	-	-	5
	Dairy, meat, wool	-	64	-	-	18	1	83
	Meat, wool	4	139	-	1	13	2	159
	Meat, wool, pelt	-	3	-	-	3	-	6
Pigs	Meat type	7	31		2	-	1	41
	Bacon type	5	17		-	-	-	22
	Lard type	5	16		5	1	-	27
Horses	Riding, meat	6	42		3	8	2	61
	Draft, pack	1	45			1	-	47
	Pony		35		1	5	-	41
Ass	Pack, draft, meat	1	7		2		1	11

[a]Source: Mason (1969).

Table 7. Total number and number of endangered breeds of domestic livestock in Europe.

Species	No. breeds[a]	No. endangered breeds[b]
Cattle	350	81
Horses	280	51
Pigs	146	30
Sheep	384	67
Goats	103	12
Total	1,263	241

Adapted from Maijala et al. (1984).

[a] 30 countries represented.
[b] Estimated.

Table 6. Endangered African cattle breeds.[a]

Breed	Location	Purpose	Reasons for decline in no.	Advantages
Mutura	Nigeria	Meat, draft	Civil war; crossbreeding; lack of interest by farmers as tractors become available	Trypanotolerant; hardy; good draft animal; low mortality
Lagune	Benin, Ivory Coast	Meat	Crossbreeding; lack of interest by farmers because of small mature size (125 kg) and low milk yields	Trypanotolerant; adapted to humid environment
Brunede l'Atlas	Morocco, Algeria, Tunisia	Meat	Crossbreeding to imported breeds	Adapted to arid zone
Mpwapwa	Tanzania	Milk,	Lack of sustained effort to develop and maintain new breed	Adapted; dual-purpose
Baria	Madagascar	Milk, meat	Crossbreeding	Adapted; dual-purpose
Creole	Mauritius	Milk, meat, draft	Crossbreeding	Adapted; multiple purpose
Kuri	Chad	Milk, meat	Political instability, numbers reduced by rinderpest and drought	High milk production; ability to float and swim in Lake Chad; tolerant of heat and humidity
Kenana	Sudan	Milk	Crossbreeding (AI) to imported dairy breeds; loss of major habitat to development scheme	High milk potential; adapted to hot, dry environment
Butana	Sudan	Milk	Crossbreeding	High milk potential; adapted to hot, semiarid environment

[a] Adapted from Adeniji (1984).

Table 9. Criteria for classifying breeds as endangered.

Species	No. active males	No. active females	
		Stable pop.	Decreasing pop.
Cattle	<20	<1,000	1,000 to 5,000
Sheep	<20	<500	500 to 1,000
Goats	<20	<500	500 to 1,000
Pigs	<20	<200	200 to 500

Adapted from Maijala et al. (1984).

Table 10. Types of databases.

Type	Information	Examples
1. Inventory	Counts (census); sometimes subclassified by age-sex-type Amount of product (meat, milk, wool, eggs, etc.); productivity per head	FAO Production Yearbook U.S. Agricultural Statistics
2. Pedigree	Records of ancestry	Breed association herdbooks Research institution records Private herd records
3. Performance	Records of performance (growth, rate, milk yield, eggs produced, etc.) Sometimes combined with records of ancestry (performance pedigrees)	Dairy Herd Improvement Assn. Research institution records Breed association sire summaries Private herd records
4. Bibliographic	Characterize appearance and performance of different needs and types of animals Increasingly computerized to facilitate organization and ease of access References and abstracts of publications	CAB: on-line bibliography with abstracts Publications: books, scientific journals

Table 11. Rules of thumb for different sources and uses of genetic diversity among beef cattle.

	Measures of relative, genetic variation, %			Relative economic importance	
Type of traits	Variation between breeds[a]	Hybrid vigor[b]	Heritability[c]	Cow herd[d]	Market[e]
Reproduction	20	10	10	5	0
Production	50	5	40	1	2
Product	10	0	50	0	1

Adapted from Trenkle and Willham (1977).

[a] Expected differences among breed types treated similarly, % of average of breed means.
[b] Advantage of crossbred over purebred parent, expressed as % of parental average.
[c] Percentage of variation within breed type that is genetic.
[d] Relative importance to commercial breeding herd.
[e] Relative importance to commercial market animals.

Table 12. Databases maintained by U.S. beef cattle associations, 1983/84.[a]

No. cattle registered (pedigree)	850,492
AI sired	29%
No. assn. maintaining performance records	
Mandatory for registration	3
Optional for registration	14
Registered with performance data	56%
No. herds in performance database	26,492
Herds with more than 50 head	21%
No. performance records in databases	
Weaning, 1983/84 only	486,712
Weaning, accumulated	4,519,945
Yearling, 1983/84 only	177,234
Yearling, accumulated	1,682,410

---

[a] Seventeen associations represented: Angus (black), Angus (red), Beefmaster, Brahman, Brangus, Charolais, Chianina, Gelbvieh, Hereford, Limousin, Maine-Anjou, Polled Hereford, Salers, Santa Gertrudis, Shorthorn, Simmental, South Devon.

Table 13. Type of traits recorded in U.S. beef cattle databases.

Traits	Database maintained by		
	Breed associations, no.	Research institutions, no.	Central test stations, no.
Ancestry (pedigree)	12	17	72
Growth and size			
Birth weight	12	16	45
Weaning weight	12	17	24
Yearling weight	12	15	57
Average daily weight gain	3	-	72
Height measurement	7	16	50
Frame score	1	1	10
Cow weight	4	11	-
Reproduction			
Gestation length	1	1	-
Calving interval	3	3	-
Calving ease	10	12	-
Weight at puberty	-	2	-
Pelvic measures	-	4	-
Scrotal circumference	6	8	52
Carcass			
Fat thickness (ultrasonic, live animal)	1	2	38
Ribeye area, fat thickness, and other carcass information	3	7	7
Other			
Feed conversion efficiency	-	2	16
Structural soundness index	-	-	1
Performance index	-	-	42
Classification score	4	11	1
Yearling grade	2	1	-
Cow condition score	2	1	-

Table 14. Budget projections for Latin American cattle data bank development in 1986[a]

Item	Cost, $U.S.
Program development, 300 hours	1,200
Computing charges, 20 hours	1,600
Data entry, 300 hours	600
Computer supplies	
20 diskettes	96
6 magnetic tapes	53
Paper, punch cards, miscellaneous	246
Research assistant in Venezuela, 1 year	7,983
Communications - telephone, telex, postage, photocopying	884
Travel and expenses for collaborators and coordinator collecting data	6,000
Total	$18,662

[a] Provided by Plasse (personal communication) for the ALPA Commission on Animal Genetic Resources.

Contract Paper No. 4

Concepts and Strategies to Maintain Domestic and Wild Animal Germplasm

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U.S. Congress

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CONCEPTS AND STRATEGIES TO MAINTAIN DOMESTIC AND WILD ANIMAL GERMPLASM

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November 18, 1985

ABSTRACT

Biological diversity at both genetic and taxonomic levels is important to the maintenance of both natural and manmade ecosystems but is being rapidly depleted by various processes of human growth and development. Genetic diversity is important for both wild and domestic animals at both the population and individual level. Natural populations require genetic variation to permit adaptation to ever-changing or changed environments. Without the capacity for adaptive evolution, extinction rate of populations and species are accelerated excessively. Genetic variation is also important for domestic stocks to permit mankind to adapt these species for changing and future human needs. Most domestic and many wild species also need genetic diversity at the individual level to sustain fertility, survival, and productivity.

Losses in biological diversity are being caused by the rapid reduction and also fragmentation of populations. Small populations lose genetic diversity primarily because of random genetic drift. Drift occurs because only a limited sample of genetic material is transmitted from one generation to the next. The rate of genetic drift is inversely related to the effective size of a population (N_e). The smaller N_e is, the faster genetic drift occurs. However, N_e is a function of the breeding structure of the population and usually is less than the census number.

Preservation of genetic diversity, therefore, entails maintenance of minimum viable populations (MVP's). MVP's depend on the amount and nature of the genetic diversity to be maintained, the length of time the diversity has to be preserved, the original level of diversity available at the outset of the program (represented as the number of founder animals), and the biological characteristics (generation time, growth rate) of the species in question. In general, MVP's of several hundreds seem necessary for most vertebrates.

Population management programs can be formulated to preserve prescribed amounts of genetic diversity for specified periods of time using living populations of a given size. Such management entails both genetic and demographic interventions. In this regard, *ex situ* captive propagation programs can be significant, even vital, for preservation of biological diversity.

The optimum structure for a captive population will usually involve some subdivision of the population into distinct subpopulations. This structure retards the rate of loss of alleles from the population as a whole and provides a measure of protection from random events that could decimate a single population. Limited immigration among populations (<1 immigrant/generation) can be used to control inbreeding within the individual lines.

Reproductive technology including cryogenic preservation of semen and embryos can appreciably augment strategies using populations of entire animals. But these techniques have been

perfected for very few species.

For wild taxa, captive propagation will ideally be used to reinforce, not replace, natural populations. Criteria for choice of taxa for propagation must include status in the wild, feasibility in captivity, and uniqueness in taxonomic and ecological terms. Rational criteria for when to intervene to preserve wild taxa must be developed.

For domestic stocks, genetic change with replacement of inferior stocks is desirable to increase the rate and efficiency of world food production. However, the current rates of replacement are excessive, and replacement often occurs without proper evaluation of replaced stocks. Maintenance of genetic diversity in domestic stocks is most important to provide support of animal production in unfavorable environments and to ensure flexibility to meet unseen future needs. Choice of stocks for conservation should be based on potential economic contribution, presence of identifiable genes with major phenotypic effects, representation of a biological extreme or evidence of a unique evolutionary history.

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INTRODUCTION

Biological diversity, at both genetic and taxonomic levels, is being rapidly depleted in both wild and domesticated animals (Myers, 1979; Soule et al., 1986; Maijala et al., 1984; FAO, 1984). Consequently, plans and programs to maintain this diversity, collectively designated animal germplasm, have become imperative. Strategies for maintenance of animal germplasm will differ for wild and domestic forms for a variety of reasons that include differences in objectives and disparities in technology available. However, programs to maintain germplasm of both wild species and domestic stocks will be based on some common principles of population biology. This paper will first consider these common fundamentals and then discuss separately the problems for wild and domestic species.

In developing strategies for maintenance of biological diversity, at both genetic and taxonomic levels, it may be useful to distinguish between conservation and preservation and between *in situ* and *ex situ* methods.

Conservation can be defined as action to sustain survival and evolution of germplasm in a "natural" habitat. Thus the term could be applied either to wild animals evolving in natural habitats under natural selection or to domestic varieties changing in their normal situations under both artificial (i.e. controlled) or natural (uncontrolled or uncontrollable) selection. Preservation can then be defined as the maintenance of germplasm outside the natural habitat, as either entire animals or germinal tissue.

By these definitions conservation and preservation largely correspond to in situ and ex situ methods. Natural populations are increasingly reduced and fragmented. Their gene pools sensu lato are becoming gene puddles and are subject to ecological and evolutionary evaporation. Consequently in situ systems and populations are requiring increasing management and are becoming in a sense "megazoos".

Strategies to maintain biological diversity therefore will need to incorporate both natural and captive populations, in situ and ex situ programs, to maintain biological diversity. Thus, much of the ensuing discussion will apply not only to captive or managed populations but also to their wild or lesser-managed counterparts.

Ex situ programs will encompass germinal tissues (sperm, eggs, embryos) and entire animals. Utilization of germinal tissues requires that the technology exists to regenerate entire animals. Currently, this technology has not been developed for most species of wild animals (Dresser, 1984). Hence, strategies for wild species must presently depend more on captive propagation employing entire animals than is the case for domestic forms where techniques utilizing germinal tissue are more available.

The two components of biological diversity, i.e. taxonomic (or in a more common but less general sense "species") and genetic, correspond to the two major problems confronting programs to maintain germplasm:

(1) selection of the taxa or stocks to be maintained.

(2) management of these taxa or stocks as populations to preserve prescribed levels of genetic variation or diversity.

Selection of taxa is a particular problem for ex situ programs because of limitations of space and resources, especially for captive propagation employing entire animals. This problem will be discussed in some detail in a later section.

Management of the taxa or stocks to be maintained must be based on principles of population biology, both genetic and demographic.

PRINCIPLES OF POPULATION BIOLOGY

Genetic Considerations

Genetic diversity is essential to the survival of populations and is frequently also important for the vigor of individuals within populations.

For populations, genetic diversity is vital to permit adaptation to changing and changed environments. Environmental change is inevitable and indeed is increasing in the modern world. Without genetic variation, adaptive evolution ceases. Maintenance of genetic diversity, therefore, preserves options for the survival of species in their natural habitat and for human efforts to improve the utility of animal resources.

For individuals within populations, genetic diversity is also positively correlated with "vigor" as measured by survival, fertility, productivity, etc., in many animal species. Rapid reductions in the genetic diversity of individuals within a population frequently are accompanied by declines in individual "vigor." This phenomenon is known as inbreeding depression. Not all natural populations manifest inbreeding depression. Some species and populations have evolved inbred population structures. However, inbreeding depression has been widely demonstrated among the larger terrestrial vertebrates that include domestic and related species and are the taxa most likely to be subjects of captive propagation programs in zoos (Falconer, 1981; Ballou and Ralls, 1982; Ralls and Ballou, 1983).

Genetic diversity is being lost from animal populations as they become reduced and fragmented. Generally, the smaller the population, the faster the loss. However, when considering genetic diversity and the loss thereof, it is important to distinguish between the population level and the individual level. Further, it will be useful to consider rather carefully the relative roles of random genetic drift and inbreeding in reduction of genetic diversity. [Crow and Kimura (1970) is a good general reference for much of the ensuing discussion of population genetics.]

In diploid organisms (including all animals), there are two copies of each gene which occupy a particular location or "locus" on the chromosomes. Alternative structural and functional forms of each gene can exist and are known as "alleles." Loci (genes) with more than one potential allele are known as "polymorphic." If only one allele occurs, the locus is considered "fixed" or "monomorphic." When an individual animal has two different alleles at a locus, it is "heterozygous" for that locus. If, instead, the two copies of the gene appear to be structurally and functionally the same, the animal is "homozygous" for that locus. A special case of homozygosity may occur when there is information on the geneology or pedigree of an animal and it is known that the two alleles at a locus may have arisen as copies of a single allele in a common ancestor of the animal's parents. When this occurs, the animal is described as "autozygous" for the locus. In any case, the particular combination of alleles that an animal carries at a locus is its "genotype."

Clearly, heterozygosity is a measure of genetic diversity at the individual level and the term "inbreeding" has been used as a measure of the loss of this genetic diversity (Wright, 1931). Formally, the "inbreeding coefficient" has been defined as the probability that both alleles at a locus in a diploid individual are identical by descent, i.e., are autozygous.

All of these concepts and terms can be extended to the population level. Thus, heterozygosity can also be a measure of the genetic diversity of a population when expressed as the number or percentage of individuals that are heterozygous for a locus. Conversely, it is also possible to describe the average homozygosity of a population for a particular locus.

Another measure of genetic diversity at the population level is the allelic diversity, i.e., the number and distribution of different alleles for each locus. In a population, there theoretically can be any number of alleles at a locus. Each allele will occur in some proportion, its "allelic, "genic" or simply "gene" frequency. Collectively, allelic diversity has been termed "heterogeneity."

Obviously, heterogeneity and heterozygosity are related. An individual or population cannot be heterozygous unless there is at least some heterogeneity or allelic diversity. (A minimum of two alleles at a locus is required.) However, rare alleles (occurring at frequencies of less than .05) do not contribute much to the average heterozygosity of a population.

In an ideal population, defined as infinite in size with random breeding among individuals (i.e., mating is "panmictic"), allelic frequencies do not change and the Hardy-Weinberg law predicts that a precise and constant proportion of homozygous and heterozygous genotypes will be maintained at each locus. However, in real populations of limited size both allelic frequencies and genotypic proportions can and do change. The more limited the population is in size, the greater this rate of change is expected to be.

The most significant and fundamental cause of these changes is "random genetic drift." Random genetic drift occurs in populations of finite (limited) size when parental gametes form the offspring of the next generation. In all populations of finite size, these offspring will carry a limited as well as random sample of the genetic material available from the parents. Consequently, a potentially and usually incomplete sample of the alleles is transmitted from one generation to the next.

The process of sampling of alleles in each generation leads to random changes in allelic frequencies and to eventual chance losses of alleles from the population. The extent of these random changes and the probability of chance losses of alleles is increased as population size declines. It can be readily demonstrated that in small populations without migration, mutation or selection, the probable ultimate effect of genetic drift is fixation of a single allele at each locus with concomitant losses of all other alleles, i.e., a complete loss of genetic diversity. [Again, Crow and Kimura (1970) is a good general reference on this and ensuing points.]

Genetic drift therefore changes gene or allelic frequencies. Indeed in the absence of mutation (spontaneous appearance of new alleles), migration (influx of new alleles from another population) or selection (elimination of alleles by differential reproductive success of animals of different genotypes), genetic drift is the only cause for loss of alleles in finite populations. As the proportions of some alleles decrease (ultimately to zero so that only one allele will remain at a locus), the proportion of heterozygotes declines (Appendix I). Thus, in small populations, average heterozygosity decreases and average homozygosity increases from the Hardy-Weinberg levels. Changes in average heterozygosity are therefore commonly used to measure the effect of random genetic drift.

The term inbreeding has also been used at the population level to denote these departures from the Hardy-Weinberg equilibrium proportions of genotypes. In this sense, the inbreeding coefficient measures the decrease in average heterozygosity of the population due to genetic drift and expressed in relation to some defined randomly mating base population.

When mating in the population is not at random, the inbreeding coefficient can increase more rapidly or, rarely, less rapidly, than expected from random genetic drift alone. If preferential matings of close relatives occur (as in linebreeding of pedigreed livestock) or if the population is subdivided (such that the number of individuals in a mating group is smaller than the total population), the rate of increase in homozygosity above Hardy-Weinberg levels is intensified. Thus, the inbreeding coefficient increases further. Indeed, the most familiar and strictest use of the term inbreeding refers to decreases in heterozygosity due to consanguineous matings.

However, it is important to appreciate that the inbreeding that arises from nonrandom mating within the population does not change allelic frequencies or cause loss of alleles from the population (Futuyama, 1979). This kind of inbreeding entails only a rearrangement of the allelic diversity among individuals so that the number of homozygous genotypes increases. Heterozygosity therefore decreases and inbreeding depression may occur. However, the effects of this nonrandom mating on heterozygosity and fitness can be reversed by a return to random mating. Indeed, nonrandom mating that avoids consanguineous reproduction will cause the average inbreeding coefficient of the population to increase by a smaller amount than predicted from random genetic drift alone. By contrast, losses in genetic diversity due to genetic drift can be counteracted only by mutation to new alleles or by migration of alleles from some gentically different populations.

In this paper, decline of genetic diversity due to random genetic drift will refer to actual loss of alleles from the population because of sampling between generations. Inbreeding will be used to refer to loss of heterozygosity and its consequences at the level of individuals whatever its cause. It will, therefore, include the irreversible losses in average heterozygosity that occur due to genetic drift as well as the reversible increases in homozygosity deriving from nonrandom matings and population subdivision.

The preceding discussion deals only with individual loci within the genome. However, most metric traits in animals are controlled by the cumulative small effects of multiple loci, i.e., are polygenic. The most convenient measure of diversity for such traits is the genetic variance. The additive portion of the genetic variance may be the most significant kind of diversity as far as selection, natural or artificial, is concerned (Falconer, 1981; Lande and Barrowclough, 1986).

In quantitative terms, the erosion of genetic variation and of heterozygosity due to random genetic drift and the increase in inbreeding are functions of population size and generally increase as the size of the population decreases. Wright (1931) originally demonstrated the quantitative relationship between the amount of original average heterozygosity remaining in generation t and the effective population size (Figure 1):

$$H_t = (1 - 1/2N_e)^t H_0. \qquad (1)$$

where H_t = the amount of original average heterozygosity remaining in generation t,

N_e = the effective population size,

t = the number of generations being considered, and

H_o = the original amount of average heterozygosity.

Analogously, the additive genetic variance remaining after t generations is:

$$\sigma^2_{G_t} = (1 - 1/2N_e)^t \sigma^2_{G_o} = (H_t/H_o)\sigma^2_{G_o} \qquad (2)$$

where H_o, H_t and t are as before and

$\sigma^2_{G_o}$ = additive genetic variance at time 0, and

$\sigma^2_{G_t}$ = additive genetic variance at time t.

The inbreeding coefficient at time t is likewise given by

$$F_t = 1 - (1 - 1/2N_e)^t$$

where F_t = the inbreeding coefficient at time t and the other variables are as before.

Thus in small populations, the average heterozygosity and additive genetic variance decrease and the level of inbreeding increases by an amount $1/2N_e$ each generation.

In comparison, the rate of decline of heterogeneity, i.e., the actual number of different alleles retained in the population, is usually faster than the decline in heterozygosity (Denniston, 1977) and is a function of the initial genic frequencies (Table 1) as well as the effective population size (see also Allendorf, 1986, and Fuerst and Maruyama, 1986).

Determination of the probability that an allele with frequency p in the base population will be lost from a population of effective size N_e after t generations is mathematically complex for small to intermediate values of t (e.g., Crow and Kimura, 1970; Denniston, 1977). However, for t values exceeding 2N (for p = .5) to 4N (for p = .1 or .9), the probability an allele will be lost (P_L), fixed (P_F) or remain segregating (P_S) is given by:

$$P_L = p - 3p(1 - p)(1 - 1/2N_e)^t$$

$$P_F = (1 - p) - 3p(1 - p)(1 - 1/2N_e)^t \qquad (4)$$

$$P_S = 6p(1 - p)(1 - 1/2N_e)^t$$

(e.g., Falconer, 1981).

All of these formulations express loss of genetic variation as a function of population size. However, the population size of relevance is not merely the total census number (N). Instead, the "effective" population size (N_e) can be defined as the size of an ideal population that would have the same rate of loss of genetic diversity due to random drift or the same rate of increase in inbreeding as the actual population (Wright, 1931; Schonewald-Cox et al., 1983; Lande and Barrowclough, 1986). Characteristics of the ideal population include: no selection, mutation, or migration; constant population size; equal numbers in each sex; and each individual has an equal opportunity to contribute progeny to the next generation (i.e., distribution of family sizes is binomial or approximately Poisson). Thus, the ratio N_e/N provides an indication of the extent to which the mating structure of the population under consideration deviates from that of an ideal population.

In real populations, the effective size N_e is a function of the number, sex ratio, and family sizes (variance in lifetime number of offspring) of the animals which reproduce, i.e., the structure and dynamics of a population. Depending on what these characteristics are, the genetically effective size of a population can vary from a small fraction, e.g., a tenth, to twice the actual number of animals.

Basically, disparities in sex ratio and differences in lifetime family sizes depress N_e below the total number of animals in the population (Appendix II; Figures 2 and 3). These relations emphasize the detriment to N_e of permitting a few individuals to dominate breeding in any generation. Overlapping generations (Lande and Barrowclough, 1986) and fluctuation in total population size also tend to depress N_e/N ratios (Appendix II). Therefore, if population size fluctuates, generations with lower N_e's exert an inordinate influence on the average N_e for the entire time period. Consequently, the most effective procedures for maximizing N_e involve equalizing numbers of males and females actually reproducing, minimizing variation in lifetime progeny number, and stabilizing population size over generations.

In natural populations, social dynamics are usually such that the genetically effective size is less than the total number. In *ex situ* programs, genetic management will potentially be able to produce higher effective sizes for the population maintained. One of the reasons captive propagation is so appropriate and important for conservation strategies is that management can maximize N_e, and hence preservation of diversity, from a given number of animals.

Subdivision of a population can importantly affect its genetically-effective size (Wright, 1943; Chesser et al., 1980; Chesser, 1983). In general, subdivision of a population into a set of sublines will increase the rate of loss of genetic diversity within each subline. However, the specific alleles that will be lost through random drift may differ among sublines. Therefore, the number of alleles that can be maintained within the entire set of sublines exceeds the number that could be maintained in a single, panmictic population of comparable size.

Yamada (1981) demonstrated that the probability that an allele will be lost from a population decreases as the number of sublines increases. Thus, for a line of size 25, the probability that an allele with an initial frequency of .10 will be lost after 50 generations is approximately .80 whereas the probability that this allele will be lost in five independent lines of size five is .90 in any one line but only $(.90)^5 = .59$ in the set of five lines. In general: $P_{loss,\ total} = (P_{loss,\ line})^n$

where $P_{loss,\ total}$ = the probability that the allele will be lost from all the lines,

$P_{loss,\ line}$ = the probability that the allele will be lost from any one line, and

n = the number of lines

Genetic theory therefore indicates that loss of alleles can be retarded by subdivision of the population. In practice, however, the extent of subdivision that is feasible will be limited by the ability of the species to tolerate the increased rates of inbreeding experienced within the sublines. Species that exist in nature in large approximately panmictic populations (a category that includes most domestic species and many of the larger vertebrate species that are most appropriate for zoos) often cope poorly with the increased rates of inbreeding that occur in small sublines. For example, Japanese quail have been shown to be extremely sensitive to the effects of inbreeding and have thwarted most attempts to rapidly develop highly inbred lines (Sittmann et al., 1966). However, P. B. Siegel (personal communication) has maintained unselected quail lines for over 36 generations at inbreeding rates of approximately 1% per generation without noticeable inbreeding depression. Thus, inbreeding rates on the order of 1% per generation may be acceptable for most species. A similar conclusion was reached by Franklin (1980).

Some species, however, possess highly subdivided population structures in nature and may accept higher inbreeding rates without undue loss in fitness (Templeton and Read, 1983). Such species would accept a more strongly subdivided population structure in captivity and would thereby allow more efficient maintenance of genetic diversity. Development of a captive management program must therefore balance the desire to subdivide the population (and thereby minimize the total number of individuals required to maintain a given level of genetic diversity) with the need to maintain a minimum population size consistent with maintenance of vigor for the species. Appendix III provides one example of how an optimum population structure might be derived.

Little consensus exists regarding the actual degree of subdivision of populations that is genetically ideal for captive management. Yamada and Kimura (1984) concluded that for most domestic livestock strains, preservation should be accomplished within a single panmictic population without subdivision. In contrast, Templeton and Read (1983) suggest that high levels of population subdivision could be utilized by active efforts to purge the deleterious effects of inbreeding from the population. These apparent differences arise from different assumptions regarding the universality of inbreeding depression in animals. Their rectification will require continued experimental work which can then be used in the context of Appendix III to derive optimum management plans.

The proceeding discussion assumed that population subdivision would be complete, with no transfer of individuals among subpopulations. In reality, however, some reciprocal migration between captive populations, or between captive and wild populations is likely. The effect of such migration is to reduce the rate of inbreeding within the subpopulation. If migrants come only from unrelated subpopulations, or from the wild, an equilibrium level of inbreeding (F_E) due to random drift will be reached within the subpopulation such that

$$F_E = 1/(4N_e m + 1) \quad . \qquad (5)$$

(e.g., Falconer, 1981) where m = the rate of migration. Thus, the maximum level of inbreeding that will be attained is a function of N_em which is the absolute number of migrants in each generation. Table 2 shows that the genetic structure of a very large panmictic population can be approached closely in a subdivided population through quite low migration rates among the subpopulations (Crow and Kimura, 1970; Ewens, 1979).

It should be kept in mind that as the extent of migration among subpopulations increases, the efficiency of maintenance of allelic diversity within the subdivided population declines. An optimum captive breeding plan would, therefore, most likely involve a subdivided population, but with the provision to use migration from other subpopulations or crossing of subpopulations to maintain acceptable levels of fitness if inbreeding depression became evident. Lacey (1985) has recently examined strategies for subdivision using computer simulation. His conclusions are that if sensitivity to inbreeding depression is not as critical as is the long-term maintenance of genetic variation within the overall captive population, subdivision should be considered and between-subpopulation migration should be kept to less than one migrant per generation.

Population subdivision will also protect against epidemics or other disasters that might annihilate any one unit or subpopulation. Logistics of management programs may also be appreciably facilitated, especially if the subpopulations are geographically localized. Even for a panmictically managed subpopulation, some geographical and institutional separation or distribution seems advisable.

Subdivisions smaller than a few tens of individuals will be particularly vulnerable to stochastic decimation and extinction caused by demographic and environmental factors (Goodman, 1985). Such demographic problems may be at least as important as sensitivity to inbreeding depression in determining how much subdivision is feasible.

Considering all of the above concepts and constraints, a rough general guideline for wild species may be maintenance of at least 10 collections but this guideline will need to be adjusted for the sociobiology of the taxa and the total size of the population.

Considering all of these factors, the size of subdivision appropriate for a particular taxon could range from a few collections to a continental program like the Species Survival Plans of the American Association of Zoological Parks and Aquariums.

Viable Population Size

From this discussion it is evident that preservation of genetic diversity requires determination of viable effective population sizes (VP). The stochasticity of nature and limitations of knowledge require that these be ranges. The lower boundary is frequently referred to as minimum viable population size (MVP). VP's and MVP's depend on at least 7 factors.

(1) the amount of genetic variation to be maintained

It is important to distinguish between equilibrium or nonequilibrium strategies (Lande and Barrowclough, 1986), i.e., attempting to completely prevent erosion of genetic variation or to merely retard loss to levels considered acceptable by some criteria. The ultimate objective should be to establish an equilibrium where mutation, migration, and selection compensate for random genetic drift. In other words, the ultimate objective should be to preserve all the genetic variation against nondeterministic (and presumably nonadaptive) forces.

This objective may not be possible or practical over the short term or for *ex situ* programs. In general, it requires twice as large a population to preserve 95% as it does 90% of the genetic diversity (as measured by average heterozygosity). An acceptable level of retention or loss must then be established. Recent analyses (Soule et al., 1986) have suggested that preserving 90% of the average heterozygosity will provide for retention of virtually all of the variation in quantitative characters and hence may be an acceptable as well as feasible objective for conservation strategies, at least in terms of captive populations and programs.

Unfortunately, adoption of definitive uniform guidelines to be generally applied to preservation of diversity is not currently possible. Much more research is needed to determine just how much diversity of various kinds (average heterozygosity, allelic diversity, quantitative variation or even chromosomal polymorphism) populations can afford to lose. Moreover, it would be preferable to know more about the population structures of all species in nature so that captive management might be adjusted accordingly.

Nevertheless, with the rate of loss of diversity that appears to be occurring on the planet, decisions are required now and there may not be resources to customize programs for thousands of endangered taxa or stocks. Thus, general guidelines may have value until or unless resources and time permit more refined and species-specific recommendations.

One reason these general guidelines may have to be subjected to reevaluation relates to the number of taxa or stocks that can be maintained with the limited capacity of facilities and resources. It is not clear at this point if more diversity or options are preserved by maintaining 100 taxa with 90% of original average heterozygosity or 200 taxa with 80%. More research and knowledge are needed.

(2) the kind of genetic diversity to be maintained

Progressively higher N_e's are required for equilibrial maintenance of quantitative variation versus average intralocus heterozygosity versus allelic diversity (i.e., heterogeneity). In terms of quantitative variation, populations of the order 10^2 to 10^3 have been suggested (Franklin, 1980; Lande and Barrowclough, 1986) for equilibrium preservation of significant amounts (i.e. > 90%) of quantitative variation. In particular, Franklin has presented an argument recommending N_e's of 500. However, equilibrial preservation of a comparable proportion of intralocus heterozygosity requires effective populations of the order of 10^5 to 10^6 (Lande and Barrowclough, 1986) and even larger Ne's are required to preserve 90% of the rarer alleles in a population (Denniston, 1977). Frankham (1980) and Hill (1982a,b) have suggested that the contribution of mutation to genetic variation in selected lines of Drosophila may be somewhat larger than had been anticipated so that directional selection response may continue indefinitely at N_e on the order of 100 (Appendix IV).

(3) the length of time this level of genetic diversity is to be preserved

The optimal and ultimate answer to this question should be indefinitely. In other words an equilibrium situation should be established. For more temporary ex situ programs, the answer might be until equilibrium conditions can be secured or restored.

With respect to wild taxa, this temporal horizon might be until the "demographic winter" is over (Soule et al., 1986), i.e., until human population and development may equilibrate at a level that permits patches of ecosystems large enough that conservation measures, and especially dependence on ex situ programs, can be relaxed. Estimates of the demographic winter's duration vary, but it could be as long as 500 to 1000 years. A further extension might be until reproductive technology permits extensive dependence on germplasm storage that can greatly reduce or eliminate loss due to random drift. If adequate research can be conducted, this technology might be generally available for wild species in 200 years (Soule et al., 1986).

For domestic populations, the temporal horizon is indefinite maintenance of sufficient variation to permit continued selection response (Hill, 1982a,b).

(4) the generation time of the taxa

Loss of genetic diversity occurs generation by generation (Figure 1), not year by year. Hence, assuming their N_e's are equal, elephants and chickens would be expected to lose exactly the same amount of genetic diversity over 10 generations. However, 10 generations for elephants is probably 250 to 300 years; for chickens it is about 10 years. Consequently for a fixed period like 200 years, the MVP for elephants can be much smaller than it is for chickens (Figure 4).

(5) the size of the founder or initial population

This fifth factor acknowledges the fact that you can't preserve more diversity than you originally possess. Establishing a managed population represents a sampling event from a source population. The animals that found a population, i.e., "the founders" may or may not contain all or most of the genetic diversity that has evolved and exists in the source population. In other words, the new population is passing through a "genetic bottleneck." A founder in this discussion is defined as an animal from the source population that actually reproduces in the conserved population. The recommended numbers assume that founders are unrelated and non-inbred. If this assumption is not valid, larger numbers will be required to achieve the desired levels of diversity.

A simple measure of the amount of the average heterozygosity or additive genetic variance of quantitative characters obtained in the sample of founders is provided by:

$$H_s = (1 - 1/2N_s)H_0 \qquad (6)$$

where H_s = the average heterozygosity in the sample,

H_0 = the average heterozygosity in the source population, and

N_s = the number of founder animals.

Thus a relatively modest number of animals (10 to 20) will contain a very high percentage of this kind of variation (>90%). As few as 10 founders can impart 95% of the average heterozygosity or additive genetic variance from the source population.

Equation 6 assumes that the founders are drawn from a single, large population. If the population is subdivided in nature, the number of founders required to obtain a given fraction of the total heterozygosity may differ somewhat from that given in equation 6, but the effects of subdivision are generally important only when subpopulations are very distinct (intrapopulation inbreeding coefficients of .30 to .50 or above). In general, founder numbers can be decreased by about one-third if the population exists as a small number (2 to 10) of very distinct subpopulations but should be increased by about one-third if a large number (50 to 100 or more) of distinct subpopulation exists (see Appendix V). If populations are subdivided, a more important consideration is to ensure that the founders represent a random sample from the entire set of subpopulation. In general, each distinct subpopulation should contribute equally to the founder group, or, if the number of founders is less than the number of subpopulations, there should be an attempt to ensure that the founders represent a random sample of available subpopulations. Genetic bottlenecks thus do not necessarily decimate genetic variation as long as the population rapidly recovers to the MVP size. However, the probability of the sampling of rare alleles will be small if founder numbers are limited. Denniston (1977) has provided calculations of the founder samples needed for various expectations that alleles of specified frequencies in the source population will be included in foundation samples.

Gilpin and Soule (1985) have performed more elaborate computations of the effect of founder size on retention of average heterozygosity and quantitative variation (Table 3). The result of these estimates is that founder sizes in the range of 20 to 30 should be adequate for most taxa. Beyond this point there are diminishing returns (Figure 5). Their model does assume that all founders are collected in a single sample at the outset of the captive program. In reality there normally could and should be some repeated inoculations of a few founders into the captive population and indeed some periodic gene flow both ways between captive and wild populations (Jones et al., 1985)

Research with experimental populations has also considered the effect of number of founders on eventual limits to selection response. Theoretical results of James (1971) suggest that with low intensity of selection, populations initiated by as few as 8 founders would be expected to express a total selection response exceeding 90% of that observed in an infinite population.

The above recommendations describe an optimal situation oriented toward an objective of preserving 90% of the original genetic diversity for 200 years. In some cases, it may not be possible to obtain enough founders to readily achieve this objective. Such difficulty should not necessarily deter development of a program for a unique or important taxon or stock that will not survive without captive propagation. In such cases, programs should attempt to conform to the optimal model as closely as possible with the founders that are available. Founder numbers in the range of 4 to 10 should not be considered prohibitive for establishment of viable populations (Templeton and Read, 1983). However, it should be realized that populations based on such small founder numbers may differ significantly in genetic composition from the source population.

(6) the fecundity or growth rate potential of the taxa

Significant amounts of genetic diversity can be lost as a population expands from its foundation to its long-term maintenance level, i.e., its carrying capacity or MVP (Nei et al., 1975). Hence, it is advantageous for the number of founders to be as close as possible to the eventual MVP and, in any case, for expansion of the population from the founder number to the MVP to be as rapid as possible. Indeed, most studies have concluded that in terms of loss of genetic variation, the number of founders is much less important than the effective population size maintained over the long term (Senner, 1980).

(7) the nature and intensity of selection that is operating

Much of the traditional treatment of loss of variation due to genetic drift assumes selection is neutral. Other forms of selection will affect required N_e's.

Selection favoring heterozygotes leads to the attainment of intermediate equilibrium gene frequencies (q_e) in infinite populations and can, under certain circumstances, retard the process of fixation of alleles by random drift in small populations. Robertson (1962) reported that heterozygote advantage may counter effects of drift for alleles with q_e between .2 and .8, but may actually enhance the probability of chance fixation for alleles with q_e below .2 or above .8. Wright (1969) reported that heterozygosity can be maintained by a heterozygote advantage of 10% over the least-favored homozygote at $N_e = 100$ for alleles with $q_e = .5$ and at Ne = 1000 for alleles with $.3 < q_e < .7$. However, maintenance of heterozygosity does not necessarily mean maintenance of multiple alleles. Wright (1969) considers a model with a potentially-infinite number of isoalleles and with a 10% selective advantage for all heterozygotes relative to all homozygotes. For N_e = 1000, 5 to 12 alleles (depending upon the mutation rate) would be maintained, but this number declines if the heterozygotes differ in selective values. Thus, selection favoring heterozygotes can preserve heterozygosity but will not necessarily preserve allelic diversity unless all heterozygotes have similar selective values.

Stabilizing selection leading to the attainment of an intermediate optimum phenotype is considered common for fitness traits (Haldane, 1954) and tends to reduce genetic variation at additive loci (Lande and Barrowclough, 1986). For populations in an artificial environment, such selection may accentuate loss of genetic variation at additive loci and is especially undesirable if fitness in the artificial environment differs substantially from fitness in the natural environment. Thus, selection in artificial environments should be avoided unless fitness in the artificial environment is closely correlated to fitness in the natural environment. Subdivision of the population into several different captive environments may help minimize loss in genetic variation from stabilizing selection, especially if genotype x environment interaction is an important source of variability.

Demographic Considerations

Demographic considerations are inextricably interrelated to genetic analysis and management. A primary concern is that the N_e's selected must be large enough to protect populations from extinction because of demographic stochasticity (Goodman, 1985).

The dynamics of populations can be analyzed and managed with 3 parameters:

A. The age and sex structure of the population at particular times, i.e. how many animals of each sex are in each age class.

B. The age and sex specific survivorships - that is, how long animals live on the average, or equivalently, what the chances are that an animal will survive to a certain age.

C. The age and sex-specific fertilities - that is, how well animals of various ages usually can be expected to reproduce.

Utilizing these parameters, a general strategy can be delineated for demographic management of populations:

(1) Establish a carrying capacity which for managed populations will be an analytically determined number that in a sense represents a compromise between maximizing the genetically effective size and providing assistance for as many taxa as feasible with the space and resources available.

(2) Expand the population to the carrying capacity as rapidly as possible to avoid loss of genetic diversity because of

lower N_e's during the growth phase.

(3) Stabilize the population at the carrying capacity to reduce oscillations that can cause extinction (Roughgarden, 1979) and avoid depression of N_e due to fluctuating size.

Stabilization of managed populations can be accomplished by some combination of managing fertilities (regulating reproduction - birth control) and survivorships (by removals or culling of animals of specified age classes). Goodman (1980) provides a general algorithm for management intervention (Appendix VI). Foose and Seal (Foose, 1983) have computed some alternative options to stabilize a captive population of tigers (Table 4).

Whatever the management program, the animals specifically selected for removal or reproduction will best be identified by genetic criteria so as to maximize preservation of genetic diversity. Naturally, both genetic and demographic management must be applied within constraints imposed by the sociobiology of the species.

The age specificity of these managerial modifications emphasizes an important principle of demographic management. It is not enough, or even possible, merely to regulate numbers. Age distributions also must be managed. Indeed, critical to demographic management of populations for maintenance of germplasm is stabilization of age structures. Constancy of number at a carrying capacity is impossible without stability of the age distributions, unless the population size is being maintained by immigrants from elsewhere. This kind of demographic subsidization will not be possible or desirable for many or most endangered species or stocks. The stable age distribution may be as important a concept as genetic diversity for management of captive populations.

A necessary, but not sufficient, trait of the stable age distribution is that each age class must contain an equal or greater number of animals than any older age class. This characteristic confers a recognizable configuration on a stable age distribution (Figure 6). Not every such pattern will represent a stable configuration for a population; the relative number in each class must represent the proportions specified by the stable age distribution defined by the survivorship and fertilities. If an age distribution does not have this kind of configuration, it is not stable. Populations without stable age distributions will behave erratically, often oscillating drastically and detrimentally.

Hence, to equilibrate a managed population at the carrying capacity, management should attempt to produce a stable age distribution at approximately this desired size. In demographic terminology, a population stable in both total numbers and age distribution is designated "stationary."

However, such demographic management must be cognizant of the genetic consequences of these manipulations (Table 5). Significant differences in both N_e and generation time result, depending on which age and sex classes are removed.

There will be many stationary configurations at the carrying capacity for a particular population in captivity, depending on how the survivorships and fertilities are managed to produce the stability. Management might then attempt to produce the best stationary configuration possible, best in this context being defined as optimization of some particular management objective or population property. In terms of preserving genetic diversity, the best configuration will constitute some configuration that optimizes N_e and generation time. Demographic management will permit sustainable harvest or yields from populations (Beddington and Taylor, 1973; Goodman, 1980).

Cryogenic Conservation of Germinal Tissue

Retention of frozen gametes or embryos is a logical procedure for preservation of genetic variation in populations that cannot be maintained by natural means. This strategy is most applicable to wild populations that could not retain sufficient habitat for maintenance but that have the potential for redevelopment of such habitat (or of suitable alternative habitat) in the future and to domestic populations that are not economically (or otherwise) viable under current conditions but are judged to have potential for future utilization. Use of frozen germinal tissue would also facilitate preservation of subspecies of wild taxa that could not be maintained in the live state (Wharton, 1984). With current technology, cryogenic storage of germinal tissue is limited almost exclusively to domestic species, but will be discussed in general terms in expectation of technological advances in other species.

Long-term storage of frozen germinal tissue represents a one-time bottleneck extending over a period that exceeds the normal generation time of the species. The goal of storage is to ensure inclusion of a high proportion of the alleles present in the parent population within the frozen store and to provide for a sample of sufficient size to allow the frozen material to be quickly expanded to a target population number consistent with future maintenance of genetic variability.

For semen stores, the probability that an allele with frequency p in the parent population is not in a semen store comprised of at least 10 doses of semen from n males is approximately $(1 - p)^{2n}$ (Smith, 1984b). Combinations of p and n that produce values of $(1 - p)^{2n}$ of .01 and .001 are shown in Table 6.

These results suggest that a semen store sufficient to generate 10 progeny of each of 40 sires would represent an exceptionally good sample of the population and that a sample sufficient to produce 10 progeny of each of 10 sires would be expected to capture over 90% of the alleles in the original population. This result is consistent with that of Smith (1984b) suggesting that storage of semen of 25 unrelated sires would result in loss of only 2% of the genetic variation in the original population. These results assume that sampled sires are noninbred, unrelated and represent a random sample of the population. These may be non-trivial assumptions in populations that tend to be subdivided in nature and suggest that sampling schemes will require careful consideration.

With respect to storage of frozen embryos, Smith (1984b) has also shown that for storage of relatively large numbers (≥10) of embryos per mating, the probability that an allele in the parent population is not in the embryo store is $(1 - p)^{4n}$ where n is the number of pair matings. Thus, if each mating will produce 10 or more viable offspring, 12 to 13 pair matings should provide a satisfactory sample of the population. While these numbers are reasonable given the assumptions, it appears unlikely that each mating will produce 10 progeny per mating with current embryo recovery and freezing techniques. A better approach would be to use equation II-1 of Appendix II to consider the possible distributions of progeny numbers from pair matings and to thereby assess the number of matings required. Let us assume a mean yield of five viable embryos per mating, and a variance (σ_k^2) of 5, 10 or 15 in number of viable embryos. For σ_k^2 = 5, 10 and 15, respectively, 13, 19 and 25 matings would be required to obtain an effective sample size of 25 (equivalent to a 2% reduction in σ_G^2). If σ_k^2 can be reduced to near 0, these results are consistent with the desired number of founder individuals recommended by Soule et al. (1986) for initiation of a breeding population. Our suggested sample size is somewhat larger than that suggested by the Council for Agricultural Science and Technology report (CAST, 1984) which recommended a store of frozen embryos that would allow production of 20 offspring per sex representing 20 or more unrelated parents.

The CAST report also notes that the size of an embryo store should be larger for less fecund species in order to minimize the inbreeding that would occur during the period of population expansion. Thus, storage of twice as many embryos (40/sex) was recommended for cattle than were suggested for sheep, goats and pigs.

Consideration of sampling procedures for storage of semen or embryos must also consider differences among individuals in ability to withstand freezing of gametes or embryos. Crawford (1981) suggests that semen from over 50% of avian males may not withstand freezing and states a heritability (h^2)for resistance of avian sperm cells to freezing of about .1. Thus, losses of alleles associated with inability to withstand cryogenic preservation of gametes and embryos may occur. Table 7 shows the fractional reduction in genetic variance in traits that are genetically correlated with freezing ability as a function of the heritability of freezing ability and its genetic correlation with other traits for species in which 50% of the males do not produce viable frozen semen. Selection for freezing ability is shown to not produce large losses in genetic variance unless the heritabilities and genetic correlations are quite large.

Cryogenic storage of germplasm stops selection within the conserved population. This result has both positive and negative implications. On the positive side, changes in gene frequency that might arise when a natural population is moved to a different, and often more controlled, environment are avoided, so that a population that is more like the original population will be available for reintroduction into the original habitat. On the other hand, if the physical or economic environment changes during the period of storage, adaptation to the changes that have occurred will have been precluded and, if the period of storage has been long, the organism may be relatively poorly equipped to function in the 'new' environment. Thus, the process of evolutionary change is suspended during the period of storage. For wild species, repeated sampling of germinal tissue from remaining wild stocks (if they continue to exist) would in part alleviate this problem. Moreover, for captive populations, it is envisioned that cryogenic stores will be used interactively with the stocks of living animals to expand their effective population sizes. Thus, there will be periodic regeneration of animals from the germinal tissues and repeated collections of germinal tissues from successive generations of living animals.

For preservation of taxa, cryogenic preservation techniques can augment, but not replace, in situ preservation efforts. Females of the taxon must exist to allow use of frozen semen, and females of the same, or a closely related, taxon must be available to gestate preserved embryos. The need for preservation of entire animals is less critical for domestic stocks because a stock can be regenerated from frozen semen by repeated backcrossing to females of other stocks.

Despite such concerns, storage of frozen gametes and embryos appears to be the method of choice for long-term conservation of germplasm resources in species for which the procedures are feasible. Problems of inbreeding and loss of genetic variability are minimized, and recovery of germplasm is expedient. Smith (1984a) has estimated that storage of frozen semen is the most economical conservation procedure if the period of conservation exceeds 5 years. Losses in adaptation due to changes in environment or to inability to benefit from selection during the period of conservation are confounding factors, however, and the net merits of various conservation strategies must ultimately be assessed in light of the goals of the program.

PROGRAMS FOR WILD TAXA

The best place to maintain biological diversity of wild taxa is in natural ecosystems. The continuing reduction and fragmentation of natural ecosystems renders this objective difficult or unfeasible for many taxa.

It is important to maintain some remnants of wild taxa or populations in a natural habitat. The wild population will permit the species to continue to be subjected to some semblance of natural selection but often will not be able to sustain the genetic diversity necessary for continued evolutionary chance, or, ultimately, for survival of the species. The captive programs can provide a reservoir of genetic variation that can be periodically infused into the natural population, or to use the imagery of G. Evelyn Hutchinson (1965), into the ecological theater so the evolutionary play can continue.

Thus, the primary purpose of ex situ programs and populations, and especially of captive propagation, is to reinforce, not replace natural populations. Captive populations are not an end in themselves. Even if the worst occurs in the immediate future, we must be optimistic that in several centuries, perhaps as long as 500 or 1000 years, the human population will stabilize and decline. It should then be possible to restore or even reconstruct ecosystems, but only if we have kept all the parts. Captive propagation is a way to help preserve options for the future. Moreover, opportunities will not always be so distant in the future.

Two scenarios are relevant. The more dismal is the situation where the natural habitat will be totally untenable for some period of time. In other words, the taxon will become extinct in the wild and recolonization would be necessary. The period of untenability could extend from a few years to several centuries, with the latter becoming more probable for many of the large vertebrates which often disappear before their habitat does.

The more hopeful, though not less challenging, scenario is that some remnants of the taxon will survive in a natural habitat but will be so diminished and fragmented that they are not viable unless reinforced by infusions from a captive gene pool. This scenario thus envisions a system of interactively managed wild and captive populations both of which are critical for the taxon's survival.

There are some who contend that reintroduction from captivity to the wild will not be feasible even if tenable wildlands are available. Among their major arguments are concerns about:

(1) possible disruption to wild populations that may still be resident in the natural habitat, and
(2) the inability of stock born and bred in captivity, perhaps for many generations, to readapt to a natural environment.

In response to these negative speculations, some limited but encouraging data can be cited from the several projects past and present that have attempted reintroductions with some success: American and European bison, Arabian oryx, golden lion tamarin (Foose, 1986). But, even if there are cases or species where formidable problems will occur, it may not be necessary or desirable to release animals. Advances in reproductive technology, e.g., artificial insemination and embryo transfer, may enable genetic material to be transferred from captive to wild populations without the complication translocating animals. Exploring such possibilities is going to be one of the most exciting areas of wildlife research in the near future.

Basically there are two major problems confronting programs for captive propagation as part of strategies to maintain animal germplasm:

(1) selection of taxa for preservation by a process of analysis and allocation to optimize usage of the limited captive habitat available, and

(2) demographic and genetic management of these taxa as biological populations.

Management of Captive Populations

A general strategy can be delineated for population management of wild taxa in captivity (Foose et al., 1986).

(1) The population should initially be expanded to the carrying capacity as rapidly as possible to insure survival of the taxon in captivity. Thereafter, population maintenance should be consistent with the constraints represented by components 2 through 10 below.

As stated earlier, the carrying capacity in captivity is an analytically determined number which is a compromise between maintaining the largest possible population for genetic reasons and allocating the limited space and resources to as many taxa as possible. However, carrying capacities will normally need to be higher than the genetically recommended minima to compensate for various stochasticities or for the fact that management may entail N_e's below the number of animals being maintained. In general, captive carrying capacities and MVP's will be several hundreds of animals. Once the established carrying capacity has been attained, the population should be stabilized at this level by demographic management (Goodman 1980; Foose and Seal, 1985).

(2) When the total panmictic effective population size is greater than 100, subdivision into units of $N_e = 50$ to 100 is recommended. For $N_e < 100$, smaller subdivisions are recommended.

(3) Inbreeding should be minimized by avoidance of matings between close relatives within subpopulations.

(4) Immigration among subpopulations should be regulated to achieve some desired compromise between panmixia and subdivision. The sensitivity of a taxon to inbreeding depression will limit the extent of genetic isolation among subpopulations. However, if subdivision is to produce meaningful reductions in genetic drift, immigration must be restricted to less than one individual per generation in each subpopulation. Immigrants into a particular subpopulation are best selected at random from the other subpopulations (unless logistic considerations dictate migration between neighboring subpopulations).

(5) Family sizes should be equalized to minimize natural selection and random genetic drift.

(6) However, if the prior captive history has produced unequal representation of founder lineages (and presumably

alleles), available information on pedigrees should be used to rectify the disparities as far as is possible. In other words, the representation of founder lineages should be adjusted to be proportional to the probable distribution of alleles surviving from the founders at the time the program of population management is initiated. A number of methods being referred to as "gene drop" programs have been developed to treat this problem (MacCluer et al., 1985; Jon Ballou, personal communication). This prescription is a more refined version of what has been a prevailing objective of "equalizing founder representations" (Foose, 1983; Seal and Foose, 1983) and recognizes the realities of allelic losses due to complicated pedigrees common in captive populations (c.f. Ryder et al., 1982; Ballou, 1984). Reproductive technology can significantly facilitate adjustment of founder representation (Ballou, 1984). Rectifying disparities in founder representation will entail a period of unequal family sizes.

(7) Once the population has expanded to the carrying capacity, generation times should be maximized to retard the rate of loss of genetic variation over absolute (versus generational) time.

(8) Continued introduction of wild genes (new founders) into the captive population is desirable, when possible, to minimize genetic changes in captivity caused by random genetic drift or natural selection in the captive environment.

(9) Reciprocally, gene flow from captivity to the wild will be determined by appropriate analyses of the condition of the natural habitat and any population(s) therein. Reintroduction mechanisms could include: artificial transfer of gametes from the captive to the wild population; release of captive/wild hybrids; and release of acclimated captive stock into unoccupied territory. These mechanisms should minimize possible problems with disruption of residual natural populations or readapting captive stock to wild conditions.

(10) Substantial changes over time in quantitative traits should perhaps be counteracted by within-family selection, maintaining equal family sizes. There should also be an attempt to select against specific deleterious traits such as recessive lethals mutations or serious pathologies (if these attain appreciable frequency in the population) by permitting some between-family selection. However, any artificial selection should be applied to captive populations of wild taxa with great caution (Frankham et al., 1986).

Selection of Taxa

The number of wild taxa whose survival may depend on captive propagation and other *ex situ* programs is large and growing. For terrestrial vertebrates where the situation is best known, it is estimated conservatively that perhaps 1500 to 2000 taxa will be endangered with extinction by 2050 (Soule et al., 1986). Among mammals, which traditionally among vertebrates have been of greatest interest and utility to mankind, it is estimated that 800 taxa may be lost without ex situ preservation programs (Table 8).

In contrast, the capacity of zoological institutions, which currently are the main facilities concerned with and capable of significant captive propagation programs, is very limited. With a few exceptions, these institutions are the established "zoos" of the world. If zoos are to utilize these resources effectively and efficiently, there must be:

(1) assessment of the space and resources, i.e., the "captive habitats," that are available for various kinds of animals, e.g., large cats, ungulates, tropical forest primates,

(2) ascertainment of what taxa are most in need of assistance from captive propagation, and

(3) allocation of the captive habitat according to some rational scheme that insures that viable populations are being accommodated.

Based on available data, Conway (1985) has calculated that the zoos of the world can accommodate 538,694 terrestrial vertebrates; 181,462 mammals; 280,357 birds; 57,725 reptiles; and 19,153 amphibians. These data are very rough and are based upon reports to International Zoo Yearbook of the numbers of specimens of each class in these institutions. Moreover, there is no information in these figures on the quality of the captive habitat available in these facilities. Elephants and snakes occupy different kinds of enclosures and have dissimilar needs. Only for the United States and Canada, where most zoos participate in the International Species Inventory System (ISIS), is there data permitting an attempt at allocation analysis.

Using Conway's crude worldwide data and assuming that captive populations of at least several hundred of each taxa designated must be maintained for long-term viability, 2000 taxa could be maintained at population sizes of 250 and 1000 taxa could be maintained at population sizes of 500.

However, because of management limitations reflecting many frailties and failures (insufficient funds, etc.), Conway estimates that perhaps only half of the calculated captive habitat will be available for significant programs. Hence, he believes that 1000 taxa represents the likely capacity of the captive habitat currently available if populations of 250 are to be maintained.

Perhaps a better appreciation can be developed from some data for North America based on ISIS records. Table 9 presents an allocation analysis for large cat taxa in U.S. and Canadian zoos. These analyses demonstrate that captive facilities can accommodate viably large populations of entire animals for only a fraction of the taxa that are or soon will be endangered.

Space is not the only resource axis defining the captive habitat. Costs for other resources are also formidable. Conway (1980, 1986) has estimated that maintenance of 2000 taxa at populations of 500 individuals for 20 years will cost $25 billion, about the amount expended by the U.S. Space Program to place humans on the moon.

Finally, any consideration of the capacity of captivity must recognize a paradox and predicament. Good population management will eventually cause every animal to be surplus for genetic and demographic reasons, i.e., animals eventually will fulfill their optimal contribution to the next generation and hence the survival of the taxon. How to contend with this surplus and still provide sanctuary for as many taxa as possible is one of most formidable problems confronting captive programs. Carrying surplus will further reduce capacity of the captive habitat which is very limited. As a consequence, many zoo professionals have suggested that euthanasia may be essential if conservation potential and responsibilities are to be fulfilled.

Clearly, captive programs are going to have to be selective until or unless their capacity can be enlarged. Selection of wild taxa for captive propagation or conservation/intervention must be based on rational criteria. As a first attempt, the zoo community in North America has formulated a set of 3 criteria:

(1) Endangerment in the wild. Certainly some cases are more urgent than others; some taxa still are not classified as endangered. However, at least for the vertebrates, most will be endangered by the middle of the next century, if not before. The best source of data on status and trends of wild taxa is the

Species Conservation Monitoring Unit (SCMU) of the International Union for the Conservation of Nature and Natural Resources. SCMU is based at Cambridge University in England, but lacks resources to perform its function in a really extensive and timely manner.

(2) Feasibility in captivity. This criterion encompasses a number of considerations. Are there enough animals available for a viable foundation? Are there facilities and staff interested in committing to an intensive management program for the taxon? Can the taxon be kept alive and reproduced in captivity? If not, are the prospects reasonable that a concerted effort will master these skills? (A spectacular example of an unfeasible taxon is the blue whale.)

(3) Uniqueness in relation to other taxa. There are six endangered subspecies of tiger. However, once one or two subspecies have been selected for captive programs in zoos, it becomes difficult to justify adding more kinds of tigers when there are so many other forms desperately in need of help, e.g., a kind of lion or a type of jaguar. The captive programs with their limited capacity must try to incorporate a gross representation of as much of the taxonomic diversity of the planet as possible. Thus, species that are the only representative of their genus, family, or order will receive particular priority.

Subspecies will be a particular problem because of the limited capacity of captive facilities. Three options seem possible:

(1) Amalgamate subspecies into superspecies or generic species (e.g., manage all subspecies of tiger from tropical mainland Asia as a single population). This strategy would be analogous to the gene pooling programs for domestic stock.

(2) Select one or two representative subspecies of a species.

(3) Attempt a nuclear population scheme as proposed by Wharton (1982,1984) that would employ frozen gametes and backcrossing in conjunction with closely related subspecies.

Unfortunately, even this rudimentary system of criteria has not yet been formulated or applied in a very systematic way. More systematic systems have been proposed. The U.S. Fish and Wildlife Service (FWS) has developed one such scheme. The Species Survival Commission (SSC) of IUCN has subjected the USFWS scheme to rather rigorous review and is attempting to formulate improved alternatives. The Captive Breeding Specialist Group of the SSC and the Species Survival Plan subcommittee of the AAZPA are also actively engaged in such endeavors.

Better criteria are needed to evaluate the genetic uniqueness of taxa, subspecies, populations and individuals. At the population level, biochemical assessment of genetic diversity most commonly involves various statistical measures of genetic distance (Nei, 1972; Chambers and Bayless, 1983). These measures are based upon electrophoretic analysis of differences in protein structure and can be used to estimate the proportions of heterozygous and polymorphic loci within populations and to estimate differences in mean gene frequencies among populations. Thus, measures of genetic distance can assist in development of sampling schemes for populations that are subdivided in nature. However, electrophoretic procedures measure only structural, not regulatory, genes and consider only fractions of the entire genome.

The DNA annealing curves being analyzed by Charles Sibley and associates at Yale University (Gould, 1985) may provide better indications of taxonomic uniqueness because they appear to compare entire genomes. Another powerful technique involves DNA sequencing using oligonucleotide primers of highly conserved regions. Other methods of value include karyotyping, mitochondrial DNA sequencing and immunological procedures (Chambers and Bayless, 1983). The best approach appears to involve diverse methodology and multiple criteria including phenotypic morphometrics as well as biochemical techniques.

At the individual level, electrophoretic procedures can assist in identifying heterozygous individuals. However, a very limited portion of the individual genome can be assessed, suggesting that regular use of these procedures for selection of genetic donors would be presumptuous.

Decisions to Intervene

There also needs to be a more rational system for deciding when to intervene for intensified management *in situ* or for establishment of ex situ programs. Recent attempts to apply decision analysis seem promising (Maguire, 1985; Maguire et al., 1986) (Figure 7).

In general, intervention has been occurring too late for many wild taxa. The California condor is a case in point.

Based on discussions of minimum viable effective population sizes above and especially N_e/N ratios evidently prevailing in natural populations, general guidelines for when to intervene can be suggested (Table 10).

Research and Resource Needs

Despite attempts by zoos to organize themselves better for captive propagation to assist conservation strategies (e.g., Species Survival Plan of the American Association of Zoological Parks and Aquariums) their resources are woefully limited. One of the main problems is that zoos are assuming global responsibilities for conservation but are still operating with local or parochial bases of support and constituencies to satisfy.

Zoos are attempting to enlarge their capacity by developing propagation facilities. But there is need to place zoos on a more independent base of support.

Establishment of a number of capacious propagation centers around the United States in representative habitats might also be advisable. (Actually almost all of the planet's habitats are approximately represented within U.S. territory.)

Concommitantly or similarly as reproductive technology is developed, a number of germinal tissue repositories (i.e. "frozen zoos") could be established.

Much of this discussion has been based on a consideration of terrestrial vertebrates because this is the best known and treated group. Aquariums and fisheries might be able to provide the same kind of support for germplasm maintenance for aquatic organisms. Herbaria and botanical gardens are attempting programs with plants. The largest lacunae in the programs is for terrestrial invertebrates. Perhaps it would be possible and appropriate to recruit universities and research facilities for this task. However, for terrestrial invertebrates there is the problem of host specificity with plants both in terms of their utility and their preservation.

Furthermore, it should be obvious from the above discussion of population biology and management that we don't yet know all the answers (or even questions) or possess all the technology to maintain animal germplasm against forces acting to deplete taxonomic and genetic variation of the planet. Thus there is need to support research on population and conservation biology and on germinal tissue technology.

PROGRAMS FOR DOMESTIC STOCKS

General Considerations

The goals of programs to maintain germplasm resources within established domestic species differ importantly from those of programs designed to maintain endangered species. Breeds of livestock do not have the same 'right' to existence that a species may be viewed to have. The process of differential propagation of alleles and change in the genetic composition of the population is an integral part of selection for economic utility within domestic populations. Thus, change in the genetic structure of domestic population with replacement of inferior subpopulations by superior strains is an accepted and desirable occurrence.

Traditionally, the process of breed replacement has proceeded on an evolutionary time scale with gradual changes in breed composition and with provision for maintenance of a wealth of local populations. More recently, however, emergence of multinational breeding companies in poultry and swine, widespread use of artificial insemination and intensified sire selection in dairy cattle, and enhanced opportunities for dissemination of germplasm throughout the world has accelerated the pace of breed replacement. In addition, increased standardization of production, marketing and recording procedures for poultry, swine and dairy cattle in developed countries of the temperate zone have increasingly promoted replacement of local breeds with populations having a national or international base. In developing countries, efforts to achieve quantum increases in food production have prompted widespread crossbreeding to so-called 'improved' temperate strains that excel in one or more of the primary production traits but are often not well adapted to the overall conditions of the country. Only recently has the need for comprehensive evaluation of the total performance of imported stocks begun to be recognized in developing countries. Unfortunately, serious dilution of original stocks may have already occurred. Thus, it is not the process of breed replacement that is of concern, but the rate of breed replacement and the danger that useful breeds may be discarded before they can be fairly evaluated.

The process of breed replacement is of particular concern in light of likely rapid technical advances in molecular biology and genetics. The capacity to extract desirable genes or gene complexes from less-productive types and to insert them into more productive types could have important implications for designing superior stocks for specific environmental conditions. Unfortunately, our knowledge of the genome in domestic animals is rudimentary. We do not know if adaptive factors such as heat tolerance and disease resistance are controlled by many or few genes and have no basis as yet to assess potential single-gene contributions of local stocks.

Forms of Conservation

Both Simon (1984) and Smith (1984b) discussed alternate forms of conservation and noted that the form of conservation depends upon the objectives of the conservation program. These forms include:

(1) Live animals reproducing distinct populations with or without selection.

(2) Gene pools of live animals in which individuals from many stocks are combined in single large interbreeding populations. Ideally, such populations conserve genes by amalgamation of alleles from several populations into a single pool from which they can be recovered as needed. Use of gene pools facilitates maintenance and recovery of mutant alleles with visible effects, but results in losses of multiple-locus epistatic (gene interaction) combinations present in original stocks. From a pragmatic standpoint, use of gene pools appears most useful for conservation of genetic material from locally-adapted stocks with similar performance levels. If conservation of alleles is the goal, gene pools should not be selected. However, if development of superior, locally-adapted stocks is the goal, development of a gene pool population with subsequent selection for performance in the native environment appears appropriate.

(3) Frozen gametes (primarily semen at this time) or embryos. As noted by Smith (1984b), the choice of storage as semen or

embryos is dictated by whether the goal of the program is the conservation of genes (as in semen or gene pools) or genotypes (as in embryos or pure lines). If the conserved stock is ultimately to be used as a pure line, if epistatic gene combinations are important or if extrachromosomal (mitochondrial) genes are important, embryos should be stored to allow regeneration of the original stock in a single generation. In contrast, reconstitution of a stock from frozen semen requires repeated backcrosses of stored semen on currently-available females. Three to five generations are required as the percentage of genes from the conserved populations increases from 1/2 to 3/4, 7/8, 15/16 and 31/32 with repeated backcrossing. However, because selection ceases in cryogenically-conserved populations but continues in primary populations, it appears unlikely that a conserved population would supplant a primary population. A more reasonable scenario would involve changes in production environment or market demands that result in a need for a partial influx of genetic material from a conserved stock into a primary stock. A composite population carrying 25 to 50% of the genes of the conserved population with the remainder from the primary population appears to be the most likely vehicle for utilization of conserved germplasm and could be generated more quickly with frozen semen. A reasonable compromise (Smith, 1984b) would involve primary storage of frozen semen but with small additional stores of frozen embryos (female only if possible) to facilitate multiplication of purebred stocks.

Goals of Conservation in Domestic Species

Simon (1984) outlined possible objectives for programs to maintain genetic diversity in domestic animals.

(1) Maintenance for educational and emotional reasons as an expression of man's heritage and culture. This situation is analogous to that found in zoo species. Considerations of population structure would be the same as those discussed elsewhere in this report for zoo species.

(2) Maintenance for animal production under unfavorable conditions. This area is often cited as the most pressing reason for conservation of animal germplasm (CAST, 1984). Needs center around an increased cognizance that stocks produced by intensive selection for one or two primary production traits (such as milk, wool or egg production) in temperate environments are unlikely to be suitable for use in developing countries without major (and often economically unfeasible) changes in the level of nutrition and management in those countries. Instead, a blending of 'improved' and indigenous stocks through systematic crossbreeding and(or) selection within populations derived from a crossbred foundation would be preferable to allow joint utilization of genes for improved production and for adaptation to the native environment. Many examples exist of situations in which crosses

of imported and indigenous strains are superior to either parent strain (Madalena, 1984; DeAlba and Kennedy, 1984).

Thus, a strategy involving evaluation of native stocks as pure lines and in crosses and development of optimum breeding strategies must supplant the strategy of simple breed replacement with so-called improved types. Such a strategy presumes conservation of genes from desirable indigenous breeds. This gene conservation could occur within selected gene pools formed by combining indigenous and improved stocks, so long as the environment of the selected stock is representative of the commercial industry of the country. Indeed, this strategy may be most appropriate for meeting the joint demands of gene conservation and improvement of commercial stocks. However, conservation of the indigenous parent stocks is also important. If the stock is being used as a part of an on-going crossing program, maintenance of pure lines is required and selection for overall economic merit within the lines is indicated. If the indigenous population is to be replaced with a crossbred population, maintenance of indigenous parent stock could be achieved by storage of semen or embryos. However, if this route is taken, it should be realized that cessation of selection within the parent stock will make it progressively more difficult for the conserved stock to compete with the commercial stock as the period of storage increases.

Thus, for maintenance of genetic variation to support animal production under unfavorable conditions, emphasis should be placed on testing and utilization of indigenous stocks and upon selection to improve indigenous stocks in their native environment. Cryogenic storage should be used primarily to conserve stocks that cannot be expeditiously evaluated now and to provide a measure of insurance in situations where breed replacement appears desirable.

(3) Maintenance in order to quickly meet unforeseen future germplasm requirements. Movement in developed countries away from local markets and toward national and international markets with concomitant increased product standardization (especially in poultry and dairy cattle) has resulted in a narrowing of selection goals and an increase in selection intensity. This trend has led to improvements in rate of selection response and overall genetic merit of the population given current goals, but concern exists that breed variation needed to accommodate potential future changes in production goals may be lost. Conservation of alternative stocks is therefore desirable. Since conservation is predicated upon eventual resurgence of the stock, conservation of breeding populations with selection will probably be required to allow such stocks to maintain a competitive position, although supplemental semen storage to maintain genetic variability may also be advised in particularly threatened stocks. Selection should be designed to emphasize the biological traits that motivated conservation, not to attempt to allow the

stock to compete directly with the primary stock in current markets.

Stocks which represent true biological extremes are also worthy of conservation to meet unforeseen future goals. Such stocks are a particularly valuable genetic resource, and, because of their extreme performance in specific traits, may have utility outside their environment of origin. For example, prolific Finnish Landrace sheep (Goot and Maijala, 1977) have been used as a component of crossbreeding systems throughout the world. Stocks such as these also have potential for use in biological research and as material for future use in genetic engineering. Propagation of such stocks in live form to allow study and evaluation in breeding systems is desirable, but cryogenic preservation may also be considered if propagation of live stocks is not feasible under current conditions. The suggestion of Land (1981) that stocks should be subjected to intense single-trait selection for high and low performance in individual traits is also of interest and would be expected to enhance rather than merely conserve genetic variation among stocks.

(4) Maintenance to extend man's knowledge of animal biology. The opportunities to use extreme stocks to study metabolic and physiological processes transcend the need of commercial agriculture alone. Conservation of such stocks becomes a priority for society as a whole.

(5) Maintenance of reserve populations to overcome possible

selection limits within present breeding populations. Theoretical work by Robertson (1960) on the theory of selection limits and a review of limits of artificial selection response in laboratory populations by Al-Murrani (1974) both suggest that long-term selection for a single trait is expected to result in eventual cessation of selection response. King (1981) assessed evidence for exhaustion of genetic variation in livestock populations and concluded that meaningful reductions in genetic variation and selection response may have occurred in egg-type poultry but were not evident elsewhere. King (1981) also noted that exhaustion of genetic variation may be retarded by the multiple-trait selection criteria appropriate in economically-important species. This is especially likely in beef cattle and sheep (our most extensively-managed species) where net economic merit is complex and where genotype x environment interactions (which tend to favor different genetic types in different environments) are prevalent. Thus, exhaustion of genetic variance due to continued selection does not appear to be a major problem in most of our primary livestock populations.

Still, in poultry, dairy cattle and swine (our most intensively-managed species), potential future depletion of genetic variation is not inconceivable. Selection plateaus have been observed for egg production in chickens (Dickerson, 1955; King, 1981) but have not normally been accompanied by apparent exhaustion of additive genetic variance (Dickerson, 1955). In

Holstein dairy cattle, Mirande and VanVleck (1985) reported apparent declines in genetic variance for milk yield between 1960 and 1982, but this pattern may be attributable to increased pedigree selection of progeny-tested bulls. No indication of loss in selection response for milk yield in Holsteins was observed between 1960 and 1979 (VanVleck et al., 1985). Young (1984) reported that the average inbreeding coefficient in the Holstein breed in 1976 was only 3.8%. However, the average relationship within the breed increased from 3.4% in 1931 to 7.9% in 1976, suggesting corresponding likely future increases in inbreeding.

A reasonable strategy in intensively-managed species might involve periodic sampling and preservation of frozen semen or embryos from such stocks. Such a program might be invoked for stocks that: (1) represent the primary production stock within a given class of livestock, (2) are exhibiting substantial rates of genetic change (≥ 1% per generation) and (3) are being maintained at limited effective population sizes (N_e < 200; usually due to intense sire selection). Retention of limited quantities of semen from dairy bulls that have been removed from service is practiced in some commercial artificial insemination organizations (P. D. Miller, personal communication), but does not represent an industry policy. In the poultry industry, several long-term randombred control stocks exist (Somes, 1984) and provide a similar reservoir of genetic variation.

Choice of Stocks for Conservation

The ultimate conservation of all readily-identifiable stocks of domestic animals is not possible or necessarily desirable. In a survey of animal genetic resources, Maijala et al. (1984) identified over 700 unique strains of cattle, sheep, pigs and horses in Europe alone and categorized 241 of these stocks as endangered. In such a situation, public policy supporting maintenance of all such stocks clearly would be unmanageable. Thus, decisions will have to be made regarding the stocks that are primary candidates for conservation.

Several criteria exist for assessing the 'uniqueness' of a stock in relation to other candidates for conservation. From an objective, evolutionary standpoint, the concept of genetic distance (Smith, 1977) can be used to assess the genetic similarity of stocks on the basis of differences in gene frequencies at polymorphic loci within a group of stocks. Such estimates can be used to evaluate the extent of genetic relationship among stocks. However, artificial selection can produce large biological differences (e.g., high versus low milk production) within related stocks. Thus, as noted by Simon (1984): "an estimate of genetic distance for two specific breeds is not a fully sufficient criterion to select the more valuable breed for conservation." However, estimates of genetic distance can insure that a stock with a unique evolutionary history is not discarded based on phenotypic performance alone.

In the context of the current assessment, the following outline for choosing stocks for conservation is proposed.

I. Stocks should be evaluated in their native environment and management system as pure lines and in crosses with other indigenous and improved stocks. This is a difficult and expensive phase, but extremely important.

II. On the basis of this evaluation, stocks should be characterized as:

   A. Useful under current economic conditions. Stocks of this sort should be integrated into the production system as quickly as possible such that their genetic material is utilized in pure lines, crosses or selected gene pools as appropriate. Pure lines should be maintained with selection for net merit under the primary production system or preserved cryogenically if maintenance as a pure line is impossible.

   B. Viable under current economic conditions in relation to other indigenous types, but inferior (in pure line or in crosses) to improved (exotic) types; no obvious biological extreme or major gene. Germplasm preservation in such populations could take two forms:

      1. Preservation of frozen semen or embryos to prevent total loss of the germplasm and as insurance during the period of breed replacement with the improved type.

2. Maintenance as pure lines or gene pools for their cultural-historical value at the option of local governments and producers.

A dual philosophy is in order here. A unique population should not be discarded until its inferiority is documented, but preservation should not hinder the utilization of improved stocks.

C. Not competitive under current economic conditions; possesses an extreme phenotype for one or more traits or carries a major gene. Such stocks should be conserved cryogenically or as pure lines. Utilization for research should be encouraged and selection to intensify the extreme phenotype should be considered.

D. Not competitive with existing adapted types; not a biological extreme; no major genes for production traits. No particular efforts should be made to conserve such stocks unless they can be documented (by genetic distance) to be unique in their genetic origin. Stocks could move from II.B to II.D as imported stocks prove themselves.

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APPENDIX I
Effect of Genetic Drift on Heterozygosity in Random-Mating Populations

For a locus with two alternative alleles, A_1 and A_2, the frequency of the A_1 allele may be represented as p ($0 \le p \le 1$) and the frequency of the A_2 allele is therefore $1 - p$. By the Hardy-Weinberg law, the expected genotypic array in a random-mating population of infinite size with no selection, mutation or migration is:

Genotype	Frequency
A_1A_1	p^2
A_1A_2	$2p(1 - p)$
A_2A_2	$(1 - p)^2$

where $[p^2 + 2p(1 - p) + (1 - p)^2] = 1$. The frequency of A_1A_2 heterozygotes is $2p(1 - p)$ and will be maximum at $p = .5$. As p approaches 0 or 1, the value of $2p(1 - p)$, i.e., the frequency of heterozygotes, will decline. Thus, genetic drift, which promotes chance fixation of either A_1 or A_2, will act to reduce the frequency of A_1A_2 heterozygotes. Note that this derivation can be straightforwardly extended to any number of alleles.

APPENDIX II

Calculation of the Effective Population Size

Mathematical expressions of the effective population number (N_e) have been derived for a variety of special population structures and will be reviewed in this appendix.

A. Unequal number of breeding males and females.

$$N_e = (4MF)/(M + F) \qquad \text{(II-1)}$$

where M is the number of males and F is the number of females (e.g., Crow and Kimura, 1970). N_e is primarily controlled by the sex (usually male) present in smallest numbers. For example, if M = 10 and F = 100, the total census number is 110 but $N_e = 36$.

B. Fluctuation in population number across generations. N_e is given by the harmonic mean population number (e.g., Crow and Kimura, 1970):

$$1/N_e = [\sum_{i=1}^{t} (1/N_i)/t] \qquad \text{(II-2)}$$

where N_i is the population number in each of t generations. N_e is seriously reduced by periods of small N. If $N_i = 100$ in nine of 10 generations but is reduced to $N_i = 10$ in one generation, $N_e = 53$.

C. Nonrandom distribution of progeny numbers. In an ideal population, each parent is assumed to be equally likely to contribute progeny to the next generation. This implies a Poisson distribution of family sizes with the variance in family size (σ_k^2) equal to the mean family size, k (which is equal to two in a population of constant size). However, in many populations σ_k^2 exceeds k. Mathematically:

$$N_e = 2N/[1 + (\sigma_k^2/k)] \qquad \text{(II-3)}$$

where N is the actual population number (Crow and Morton, 1955). Note also that in managed populations, if each mated pair contributes exactly two offspring to the next generation, $\sigma_k^2 = 0$ and $N_e = 2N$.

D. Joint effects of sex ratio and distribution of family size. Hill (1972) demonstrated that in this situation:

$$\begin{aligned}(1/N_e) = {} & (1/16M)[2 + \sigma_{mm}^2 + (2M/F)\sigma_{mm,mf} + (M/F)^2\sigma_{mf}^2] + \\ & (1/16F)[2 + (F/M)^2\sigma_{fm}^2 + (2F/M)\sigma_{fm,ff} + \sigma_{ff}^2] \\ & \text{(II-4)}\end{aligned}$$

where M and F are the numbers of males and females, respectively, σ_m^2 is the variance among sires in number of male progeny, σ_{mf}^2 is the variance among sires in the number of female progeny and $\sigma_{mm,mf}$ is the covariance among sires in numbers of male and female progeny. The quantities σ_{fm}^2, σ_{ff}^2 and $\sigma_{fm,ff}$ are comparable values for dam progeny numbers.

In a simplified derivation, Gowe et al. (1959) demonstrated that if each sire produces exactly one male and F/M daughters and each dam produces exactly one daughter and a son with probability M/F, equation II-4 becomes:

$$(1/N_e) = (3/16M) + (1/16F). \qquad \text{(II-5)}$$

Smith (1976) also modified equation II-4 to estimate the effect of failure of sires and dams to produce appropriate replacements such that:

$$(1/N_e) = (1/16M)(4.17 - 2p_m) + (1/16F)(4.17 - 2p_f) \quad (II-6)$$

where p is the probability that a male or female will survive and produce the required number of progeny.

APPENDIX III

A Procedure for Developing an Optimal Level of Subdivision Within a Captive Population

A decision regarding the extent to which population subdivision is indicated can be based on the following logic:

A. Develop a goal for the amount of genetic diversity that is to be conserved. This can be expressed in a variety of ways, but for purposes of example, let it be described in terms of the percentage of the initial heterozygosity that is to be maintained for a given number of generations. Let us assume an arbitrary goal of maintenance of 90% of the initial heterozygosity for 100 generations. From equation 1 of the text, this would require a panmictic (non-subdivided) population of effective size $N_e = 356$.

B. Let the maximum rate of inbreeding that is consistent with the continued fitness of the species (ΔF_{MAX}) be arbitrarily set at 1.5%/generation. In reality, this value would have to be determined separately for each species. If $\Delta F_{MAX} = .015$/generation, then the minimum effective size of a subline (N_S) is given as:

$$\Delta F_{MAX} = 1/2N_S = .015$$

such that $N_S = 33.3$. After 100 generations of random mating, only 22% of the initial heterozygosity is expected to be retained for any single line with $N_S = 33.3$.

C. For a population composed of k sublines, the heterozygosity expressed in a composite population derived by crossing of the sublines with subsequent random mating is directly proportional to the number of sublines. Such a population would be a reasonable one for reintroduction of a species into the wild and would have

$$1 - H_t'/t/H_o = (1/k)(1 - H_t/H_o) = (1/k)[1 - (1 - 1/2N_S)^t]$$

where H_o is the original heterozygosity, H_t is the remaining heterozygosity within a subline at generation t and H_t' is the heterozygosity within the composite population derived by crossing the lines. For $N_S = 33.3$ and $t = 100$,

$$1 - H_t'/H_o = (1/k)[1 - (1 - 1/66.6)^{100}].$$

If we set $(1 - H_t/H_o)$ equal to .10 (the goal of the conservation program), $k = 7.80$. Thus for 8 lines of size $N_s = 33.3$, the loss in heterozygosity would be restricted to 9.7%. A total effective population size of 266.4 would be required for the subdivided population and would compare to a value of $N_e = 356$ which would be required to achieve the same result in a totally panmictic population.

APPENDIX IV

Contribution of Mutation to Maintenance of Genetic Variation

Maintenance of genetic variation for an indefinite period appears to be a daunting task if the number of generations is large (≥100) and if we ignore possible countervailing effects of mutation, natural selection and migration (Figure 1). However it appears clear that these factors cannot be ignored indefinitely. Mutation acts to counter effects of small population size by continual creation of new alleles within the population. Thus, in any population, an equilibrium state must eventually be attained at which the chance losses of alleles in the population is exactly balanced by the generation of new genetic variation by mutation. For individual loci, it has generally been considered that the average inbreeding coefficient cannot indefinitely be held below .20 unless N_e approaches the inverse of the mutation rate (e.g., Crow and Kimura, 1970). Thus N_e would have to be on the order of 10^5 and would therefore be far larger than practical for managed populations.

Experimental results with Drosophila, however, suggest that for the quantitative trait of abdominal bristle number the contribution of mutation to the total genetic variance in each generation may be as large as $10^{-3}\sigma_e^2$ where σ_e^2 is the environmental variance within an inbred line (Mather and Wigan, 1942; Clayton and Robertson, 1955; Lande, 1976). Thus, the increase in genetic variation from mutation would be equivalent to $10^{-3}\sigma^2_{G_o}(1-h^2)/h^2$ per generation where $\sigma^2_{G_o}$ is the genetic variance in an outbred population and h^2 is heritability, i.e., $h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_e)$. This value can be contrasted to the decrease in genetic variance from genetic drift of $(1/2N_e)\sigma^2_{G_t}$ in each generation where $\sigma^2_{G_t} = (1 - F_{t-1})\sigma^2_{G_o}$ such that at equilibrium:

$$10^{-3}(1 - h^2)/h^2 = (1/2N_e)(1 - F).$$

For $h^2 = .33$ (similar to that of bristle number in Drosophila), this relationship between N_e and equilibrium F suggests that F can be held at 0 for $N_e = 250$ and is not expected to increase above .30 for $N_e = 175$. If, by manipulation of progeny numbers, N_e can be increased to 1.75 N, these results suggest that census population numbers (with equal numbers per sex) of 100 to 143 may well be sufficient to allow response to directional selection within the population to continue indefinitely.

APPENDIX V

Effect of Subdivision in Natural Populations on the Number of Founders Required to Develop a Captive Population

The number of individuals required to transfer a specified amount of the existing genetic diversity from a natural population to a captive population will depend in part upon the population structure in nature. This appendix will outline the relationship between founder number and population structure in nature.

A. All calculations will take as their original base a presumed infinite population with heterozygosity H_o. The subpopulations found in nature are assumed to have been founded from this base. Likewise, if the population exists in nature as a single, unsubdivided population, it is presumed to have arisen initially from some conceptual infinite base population.

B. If the population is unsubdivided (panmictic) in nature, and has persisted as such for t generations with a population size of N_P, the heterozygosity in the native population relative to the presumed infinite base population (H_{P_t}/H_o) will be:

$$H_{P_t}/H_o = (1 - 1/2N_P)^t \qquad \text{(V-1)}$$

For $t = 100$ and $N_P = 10^8$, $H_{P_t}/H_o = .999999500$.

C. If the population is subdivided in nature into k subpopulations of size N_S (where $kN_S = N_P$), then the expected heterozygosity within each line relative to the infinite base population (H_{S_t}/H_o) is:

$$H_{S_t}/H_o = (1 - 1/2N_S)^t \qquad \text{(V-2)}$$

and the intraline inbreeding (F_{s_t}) will be:

$$F_{S_t} = 1 - (1 - 1/2N_S)^t \qquad \text{(V-3)}$$

For $k = 10^5$ and $N_S = 1{,}000$ at $t = 100$, $H_{S_t}/H_o = .95122$ and $F_{S_t} = .04878$.

If the subdivided population were to be reconstituted by crossing all sublines at generation t and subsequently maintained as a panmictic population, the total heterozygosity in the reconstituted population (H_{T_t}/H_o) would be:

$$H_{T_t}/H_o = 1 - (1/k)[1 - (1 - 1/2N_S)^t] = 1 - F_{S_t}/k. \qquad \text{(V-4)}$$

H_T will henceforth be taken as representative of the heterozygosity contained in the subdivided natural population.

For $k = 10^5$, $N_S = 1{,}000$ and $t = 100$, $H_{T_{100}}/H_o = .999999512$.

If reconstitution by crossing involves only j lines ($j \leq k$), equation V-4 becomes:

$$H_{T_t}/H_o = 1 - (F_{S_t}/j). \qquad \text{(V-5)}$$

Thus for $k = 10^5$, $N_S = 1{,}000$, $t = 100$ and $j = 40$, $H_{T_{100}}/H_o = .99878$.

D. If N_L individuals (founders) are drawn from a panmictic natural population at generation t to initiate a captive population, the heterozygosity expected in the sample relative to that found in the natural populations at generation t (H'_t/H_{P_t}) is:

$$H'_t/H_{P_t} = 1 - 1/2N_L \qquad \text{(V-6)}$$

and relative to the presumed base population:

$$H'_t/H_o = (H'_t/H_{P_t})(H_{P_t}/H_o). \qquad \text{(V-7)}$$

For $N_L = 40$ and all other values as in the previous example, $H'_t/H_{P_t} = .9875$ and $H'_t/H_o = .9874995$.

E. If the population is subdivided in nature and at generation t, we sample m individuals from each of j lines ($j \leq k$), the heterozygosity in a sample from a single subpopulation (H'_{S_t}) relative to the subpopulation will be:

$$(H'_{S_t}/H_{S_t}) = 1 - 1/2m. \qquad \text{(V-8)}$$

Relative to the infinite base population:

$$(H'_{S_t}/H_o) = (H'_{S_t}/H_{S_t})(H_{S_t}/H_o) = (1 - 1/2m)(1 - 1/2N_S)^t$$

(V-9)

For $j = 40$ and $m = 1$ ($jm = N_L$), $H'_{S_t}/H_{S_t} = .5$ and $H'_{S_t}/H_o = .45240$.

If the captive population is formed by crossing and random mating of the individuals sampled from the subpopulations, the heterozygosity in the sample relative to the base population (H'_{T_t}/H_o) will be:

$$(H'_{T_t}/H_o = 1 - (1/j)[1 - (1 - 1/2N_S)^t(1 - 1/2m)] \qquad \text{(V-10)}$$

and heterozygosity relative to the natural population (H'_{T_t}/H_{T_t}) will be:

$$H'_{T_t}/H_{T_t} = (H'_{T_t}/H_o)/(H_{T_t}/H_o)$$

$$= \left[\frac{1 - (1/j)[1 - (1 - 1/2N_S)^t(1 - 1/2m)]}{1 - (1/k)[1 - (1 - 1/2N_S)^t]}\right] \text{(V-11)}$$

For our example with $N_L = 40$, $j = 40$, $m = 1$, $N_S = 1{,}000$, $k = 10^5$ and $t = 100$, $(H'_{T_t}/H_o) = .98689$ and $(H'_{T_t}/H_{T_t}) = .99938$. Note that $(H'_{T_t}/H_{T_t}) > (H'_t/H_{P_t})$ for this example when $mj = N_L$.

Therefore, if one can express the goals of the sampling event in terms of desired heterozygosity obtained (H), one can solve for the required number of founders in a panmictic population (N_L) or a subdivided population (mj) by using equations 6 and 11, respectively. Equation 11 can be simplified to express heterozygosity in terms of the average inbreeding of the subpopulations in nature (F_{S_t}) as:

$$H'_{T_t}/H_{T_t} = \frac{1 - (1/j)[F_{S_t} + (1 - F_{S_t})(1/2m)]}{1 - (1/k)F_{S_t}} = H' \qquad (12)$$

The objective of the sampling from a subdivided population will be to minimize the sample size, jm, required for H' to equal some target level of heterozygosity (H). Equation 12 cannot be solved directly for minimum jm, but the following numerical procedure will yield a minimum solution:

1. Set $m = 1$, $j = k$ and solve for H'.

2. If $H'<H$, increase m until $H' = H$.

3. If $H'>H$, decrease j until $H' = H$, i.e., sample only a random set of subpopulations.

Appendix table V-1 gives required founder numbers to obtain fixed amounts of heterozygosity in panmictic and subdivided populations as a function of F_S and k.

APPENDIX TABLE V-1. FOUNDER NUMBERS REQUIRED TO OBTAIN FIXED AMOUNTS OF HETEROZYGOSITY IN SUBDIVIDED POPULATIONS[a]

		5		10		50		100	
F_S	H_R	m	j	m	j	m	j	m	j
.1	.90	1	4-5	1	5	1	5-6	1	6
	.95	2	5	1	10	1	11	1	11
	.99	10	5	5	10	1	50	1	50
.3	.90	1	4-5	1	5	1	6	1	6
	.95	2	5	1	8	1	12	1	12
	.99	7-8	5	4	10	1	40	1	50
.5	.90	1	4-5	1	5	1	7	1	8
	.95	2	5	1	8	1	13	1	14
	.99	7-8	5	3	10	1	38	1	50
.7	.90	1	3-4	1	5-6	1	8	1	8
	.95	1	4-5	1	7-8	1	14	1	15
	.99	4	5	2	10	1	36	1	50
.9	.90	1	3-4	1	5-6	1	8	1	9
	.95	1	4-5	1	7	1	14	1	16
	.99	1-2	5	1	9-10	1	34	1	50

[a] Values are based on equation V-12 where $N_R = (H'_{T_t}/H_o)$ is the heterozygosity expressed in panmictic matings among the founders relative to that present in the natural population, F_S in the intraline inbreeding, k is the number of lines found in nature, j is the number of lines sampled and m is the number of individuals sampled per line.

APPENDIX VI

General Algorithm for Demographic Stabilization of a Management Population

Goodman (1980) has provided a general algorithm for stabilization of a managed population by removal (culls) of animals from specified age classes. Concommitant regulation by birth control is incorporated by the values used for fertilities in the equation. In other words, the fertilities can be managed or unmanaged (natural) values.

$$\sum_{x=1}^{w} l_x m_x \prod_{i=1}^{x-1} (1 - h_i) = 1$$

where l_x = the age specific survivorship = the probability of surviving from birth to age x.

m_x = the age specific fertility = the expected number of offspring that will be produced by a animal of age x-1 to x. (These fertilities may represent managed values produced by various birth control programs.)

h_i = the fraction of age class x removed during each time interval.

w = the oldest age class achieved by members of the population

TABLE 1. THE EXPECTED NUMBER OF ALLELES AFTER t GENERATIONS OF DRIFT IN A POPULATION OF 6 INDIVIDUALS GIVEN 3 DIFFERENT STARTING FREQUENCIES[a,b]

No. of generations	Number of alleles when:		
	$m=2$, $p=p=1/2$	$m=4$, $p=1/4$	$m=12$, $p=1/12$
0	2.00	4.00	12.00
1	1.99	3.87	7.78
2	1.99	3.55	5.88
3	1.95	3.22	4.80
4	1.91	2.94	4.08
8	1.67	2.18	2.64
12	1.48	1.77	2.04
16	1.34	1.52	1.68
20	1.24	1.36	1.44
56	1.01	1.02	1.02
	1.00	1.00	1.00

---

[a] Taken from Denniston (1977) where the expected number of alleles (n) at generation t is given by

$$1 - \sum_{j=1}^{m} \sum_{i=1}^{\infty} (2i + 1)p_j(1 - p_j)F(i + 2, 1 - i, 2, 1 - p_j)(-1)^i$$

$$\exp\,[-i(i + 1)t/4N_e]$$

where m is the number of alleles, p is the allelic frequency, F is the hypergeometric function and N_e is the effective population size.

[b] Population size is constant at 6 in this table. The number of expected alleles for each set of frequencies will increase as population size increases.

TABLE 2. RELATIONSHIP BETWEEN NUMBER OF MIGRANTS IN EACH GENERATION AND THE EQUILIBRIUM LEVEL OF INBREEDING (F)[a]

Number of migrants	Equilibrium F
1	.20
2	.11
3	.08
4	.06
5	.05
10	.02
20	.01

[a] Taken from Falconer (1981) as equilibrium $F = 1/(4N_e m + 1)$ where N_e is the effective population size, m is the migration rate and $N_e m$ is therefore the number of migrants.

TABLE 3. N_e's REQUIRED TO PRESERVE 90% INITIAL GENETIC VARIANCE FOR 200 YEARS FOR VARIANCE SETS OF FOUNDER NUMBERS, GENERATION TIMES, AND GROWTH RATES OF POPULATION[a]

Carrying capacities necessary for the retention of 90% of the initial genetic variance. The exponential growth rate is .1

	Length of Generation (years)									
# of founders	1	2	4	6	8	10	15	20	25	30
6	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—	—	32
26	—	—	—	—	—	—	—	—	—	30
28	—	—	—	—	—	—	—	—	—	29
30	—	—	—	—	—	—	—	—	43	29

Carrying capacities necessary for the retention of 90% of the initial genetic variance. The exponential growth rate is .3

	Length of Generation (years)									
# of founders	1	2	4	6	8	10	15	20	25	30
6	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—	—	39
18	—	—	—	—	—	—	—	—	—	34
20	—	—	—	—	—	—	—	112	60	32
22	—	999	999	999	642	384	137	77	50	30
24	999	999	999	582	362	243	106	66	45	30
26	999	999	768	424	274	192	92	60	43	29
28	999	999	617	348	234	164	84	56	42	29
30	999	999	538	309	208	148	79	54	40	29

Carrying capacities necessary for the retention of 90% of the initial genetic variance. The exponential growth rate is .5

	Length of Generation (years)									
# of founders	1	2	4	6	8	10	15	20	25	30
6	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	157	55
16	999	999	999	936	617	424	188	110	70	39
18	999	999	882	517	348	253	124	80	55	35
20	999	999	655	384	269	196	102	69	49	32
22	999	999	538	321	225	167	90	62	45	31
24	999	999	468	285	200	148	84	59	43	30
26	999	936	432	258	184	137	79	55	42	30
28	999	865	399	243	174	129	76	54	41	29
30	999	815	376	229	164	124	73	52	40	29

Carrying capacities necessary for the retention of 90% of the initial genetic variance. The exponential growth rate is 1

	Length of Generation (years)									
# of founders	1	2	4	6	8	10	15	20	25	30
6	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—
10	—	—	999	999	999	999	682	392	225	102
12	999	999	999	617	432	321	174	117	80	49
14	999	999	655	399	285	216	122	85	61	40
16	999	999	517	321	229	174	102	71	53	35
18	999	955	450	280	200	154	90	65	48	33
20	999	848	399	253	184	140	84	60	45	32
22	999	783	376	234	170	132	79	57	43	31
24	999	738	355	220	161	124	76	55	42	30
26	999	709	334	212	154	119	73	53	42	30
28	999	682	321	204	151	117	71	52	41	29
30	999	655	315	200	145	115	70	52	40	29

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[a] From Soule et al., 1986.

TABLE 4.

SOME OPTIONS TO ATTEMPT DEMOGRAPHIC STABILIZATION OF AMUR TIGER POPULATION AT CARRYING CAPACITY OF 250

	OPTION 1	OPTION 2	OPTION 3	OPTION 4	OPTION 5	OPTION 6	OPTION 7	OPTION 8	OPTION 9
IF REPRODUCTION IS THEN	EQUIVALENT TO 1966-80 LITTER SIZE 2.43 CUBS	ADJUSTED TO COMPENSATE FOR MORTALITY EQUALLY DISTRIBUTED OVER AGES	ONE LITTER 2.43 CUBS AT ANY AGE	ONE LITTER 3 CUBS AT AGE 4	ONE LITTER 3 CUBS AT AGE 5	TWO LITTERS 2.43 CUBS EACH AT AGES 5 & 10	TWO LITTERS 2.43 CUBS EACH AT AGES 4 &7	THREE LITTERS 2.43 CUBS EACH AT AGES 4, 8, 12	LITTER OF 2.43 CUBS ALTERNATE YEARS
THE REMOVAL OF 0-1 YEAR OLDS REQUIRED FOR STABILITY IS	46%	0%	THIS LEVEL OF REPRODUCTION APPEARS INSUFFICIENT	PROBABLY 0%	THIS LEVEL OF REPRODUCTION APPEARS INSUFFICIENT	30%	35%	53%	72%
-OR- REMOVAL FROM EACH AGE CLASS REQUIRED FOR STABILITY IS	7%	0%	TO SUSTAIN POPULATION WITH PRESENT MORTALITY	PROBABLY 0%	TO SUSTAIN POPULATION WITH PRESENT MORTALITY	4.85%	7.75%	9.5%	15.5%
GENERATION TIME	7.75 YEARS	7 YEARS	WILL VARY	4 YEARS	5 YEARS	7 YEARS	7 YEARS	7.5 YEARS	7.5 YEARS

(From Foose, 1983.)

TABLE 5. EFFECT OF REMOVAL ON N_e AND GENERATION TIME IN MOOSE (Alces)

Removal Regime	N_e/N	Generation (years)
Equal removal from all age classes	.426	4.8
Equal removal from adult age classes	.415	4.2
Removal from 0-1 age class	.396	9.9
Removal from 1-2 age class	.289	7.6
A variable removal from each adult age class	.368	6.5
A variable removal from each age class	.302	7.5

(From Ryman et al., 1981.)

TABLE 6. NUMBER OF SIRES THAT MUST BE SAMPLED TO REDUCE THE PROBABILITY OF AN ALLELE WITH FREQUENCY P BEING EXCLUDED FROM A SEMEN STORE TO BELOW .01 OR .001

Gene frequency	Number of sires at probability .01	.001
.01	229	344
.05	45	67
.10	22	33
.25	8	12
.50	4	5
.75	2	3
.90	1	2

TABLE 7. FRACTIONAL REDUCTION IN GENETIC VARIANCE IN CORRELATED TRAITS FROM SELECTION FOR ABILITY TO FREEZE GAMETES OR EMBRYOS[a]

Heritability of freezing ability	Percentage reduction in genetic variance for genetic correlation of:			
	.25	.50	.75	1.0
.10	0.2	0.8	1.8	3.2
.50	1.0	4.0	9.0	15.9

[a] The fractional reduction in the genetic variance is given as $h^2 r_G i(i - z)$ where h^2 is heritability, r_G is the genetic correlation, i is the mean of the selected sample on a standard normal curve and z is the point of truncation selection on a standard normal curve. Tabular values assume that only 50% of the individuals produce viable gametes or embryos ($i = .798$; $z = .3989$).

TABLE 8. NUMBER OF MAMMALIAN SPECIES EXPECTED TO BE CRITICALLY ENDANGERED BY THE MIDDLE OF THE 21ST CENTURY

Order	Number of recognized species	Number of vulnerable species that may require captive breeding
Monotremata	4	
Marsupicarnivora	123	2
Peramelina (bandicoots)	18	
Paucituberculata (shrew-like)	7	
Diprotodonta (herbivores)	101	
Insectivora	409	
Dermoptera (colugos)	2	2
Chiroptera	~900	100
Primates	160	160
Edentata (anteaters, sloths)	25	10
Pholidota (pangolins)	7	5
Mysticeta	10	2
Odontoceta (toothed whales)	65	10
Carnivora		
Canidae	35	35
Ursidae	7	2
Felidae	72	60
Others	193	100
Lagomorpha	53	?
Rodentia	~1700	100
Tubulidentata	1	1
Probroscidea	2	2
Hyracoidea	11	5
Sirenia	4	4
Perissodactyla	15	15
Artiodactyla	~172	100
		815

(From Soule et al., 1986.)

TABLE 9. CAPACITY OF ISIS CAPTIVE FACILITIES FOR LARGER FELIDS

Species	Number of extant subspecies	Number of subspecies in red data book	ISIS institutions[a]	ISIS population[b]	Number subspecies if population[c] 100	250	500
Panthera leo	11	1	97	381	4	1	1
Panthera tigris	8	8	110	450	4	2	1
Lions and tigers	19	9	120	831	8	3	2
Panthera onca	8	8	65	178	2	1	0
Panthera pardus	15	15	72	246	2	1	0
Panthera uncia	1	1	35	128	1	0	0
Felis concolor	29	2	69	173	2	1	0
"Intermediate" cats	53	26	N.C.	725	7	3	1
Neofelis nebulosa	4	4	20	63	1	0	0
Acinonyx jubatus	6	6	32	166	1	0	0
"Other" large felids	10	10	N.C.	229	2	1	0
TOTALS	82	45		1785	18	7	3

---

[a] The number of institutions participating in the International Species Inventory System (ISIS) that maintain the species.

[b] The total population in ISIS facilities: a crude measure of the capacity of the ISIS institutions.

[c] The number of subspecies that can be accommodated if each subspecies is maintained at a population of the indicated size.

(From Foose, 1983.)

TABLE 10.

General guidelines to decide when to intervene for conservation of natural populations.

PRIORITY	POPULATION STATUS	ACTION
possibly	$N < 100,000$	at least serious surveillance of status and trends should be initiated.
probably	$N < 10,000$	well managed captive propagation programs should be established, reproductive technology research should be vigorously conducted, and germinal tissues collected for storage, while there are an adequate number of animals to use as founders, subjects, and donors.
certainly	$N < 1,000$	*ex situ* programs should be intensified while field (*in situ*) efforts are fortified for a "last stand"; *ex situ* programs are imperative.
urgently	$N < 500$	*Ex situ* programs assume at least as much importance as field (*in situ*) efforts.

It would be better to predicate these guidelines on actual N_e's rather than N's. However, effective population sizes have been and will be difficult to measure for wild populations. Not only is information on sex ratios and family sizes often insufficient, but subdivison of the population and lack of data on rates of gene flow among subpopulations will complicate estimates. Nevertheless in these cases, a quick and dirty approximation may be:

$$N_e = 4 N_m N_f / N_m + N_f$$

where N_m = the estimated number of adult males

N_f = the estimated number of adult females

FIGURE 1.

DECLINE OF GENETIC DIVERSITY FOR VARIOUS EFFECTIVE POPULATION SIZES (N_e) POSSIBLE FOR A TOTAL POPULATION (N) OF 250

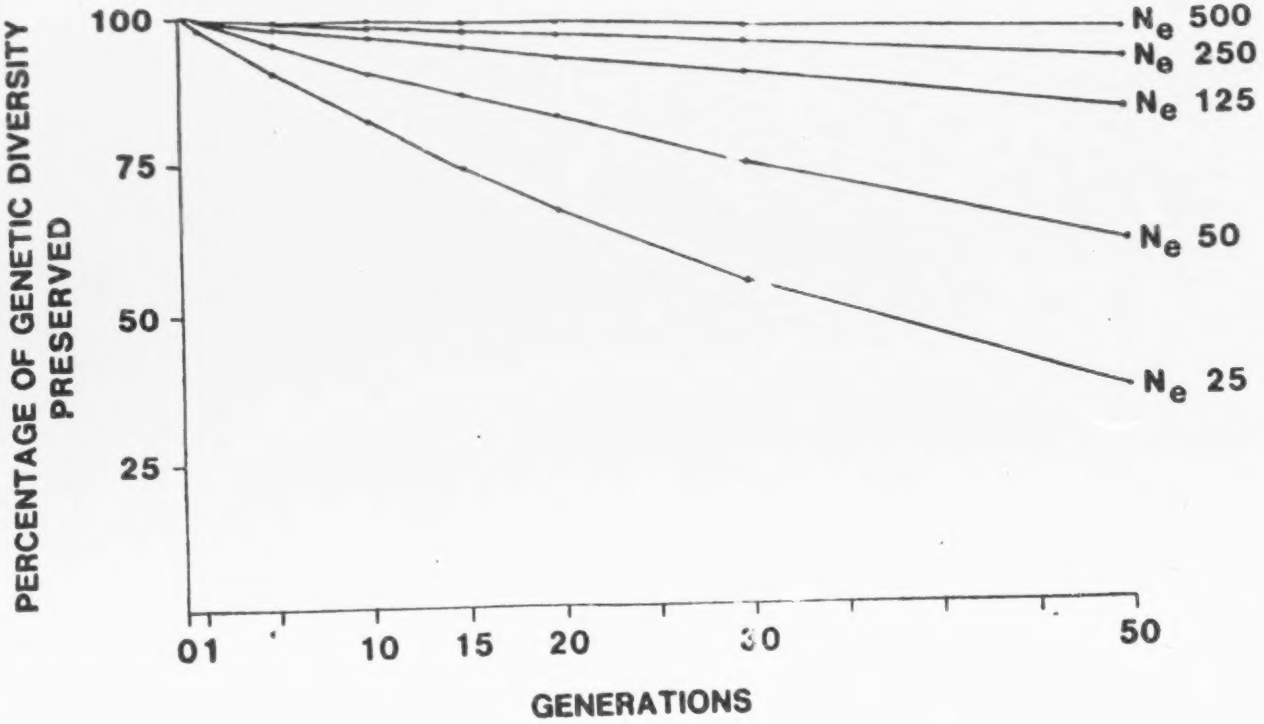


FIGURE 2.

EFFECT OF SEX RATIO ON EFFECTIVE POPULATION NUMBER

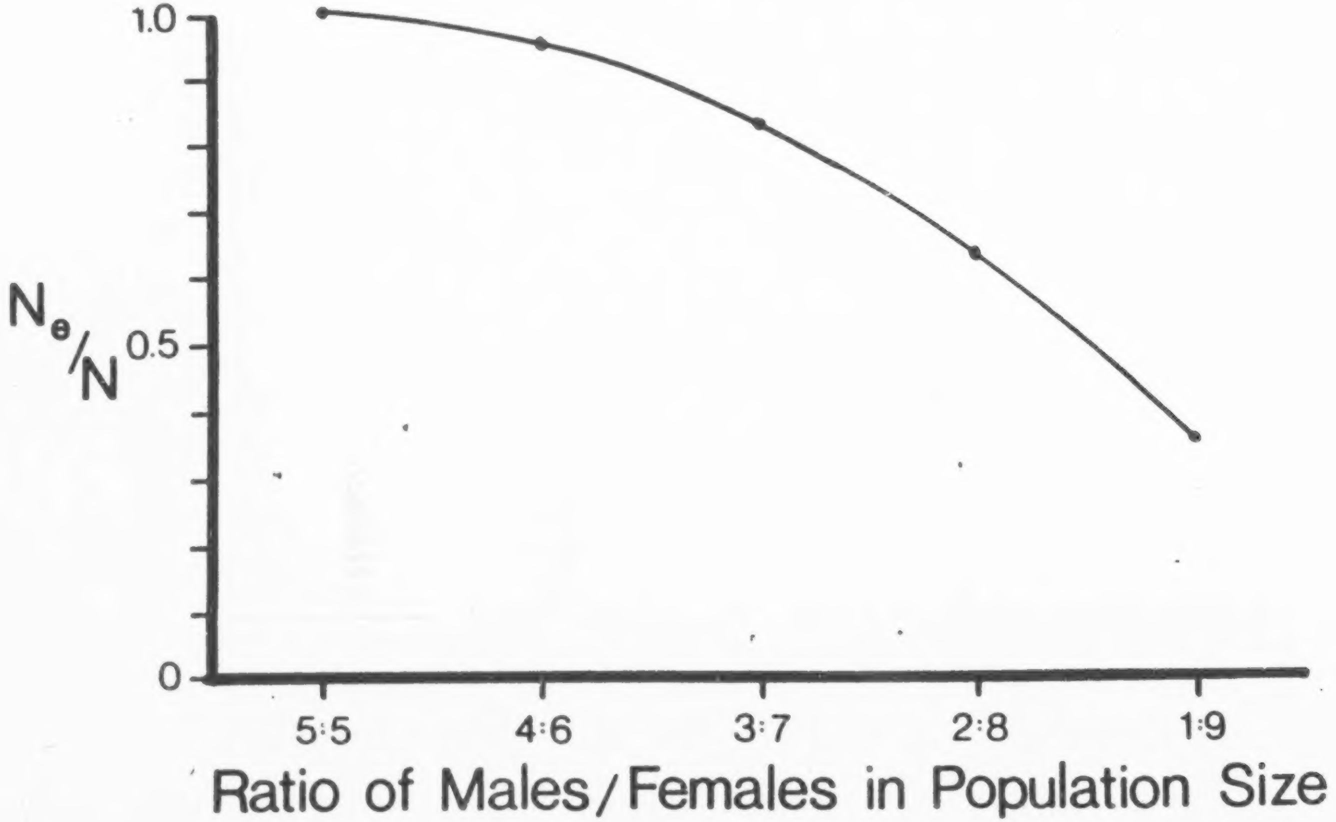


FIGURE 3.
EFFECT OF VARIANCE IN FAMILY SIZE
ON EFFECTIVE POPULATION NUMBER

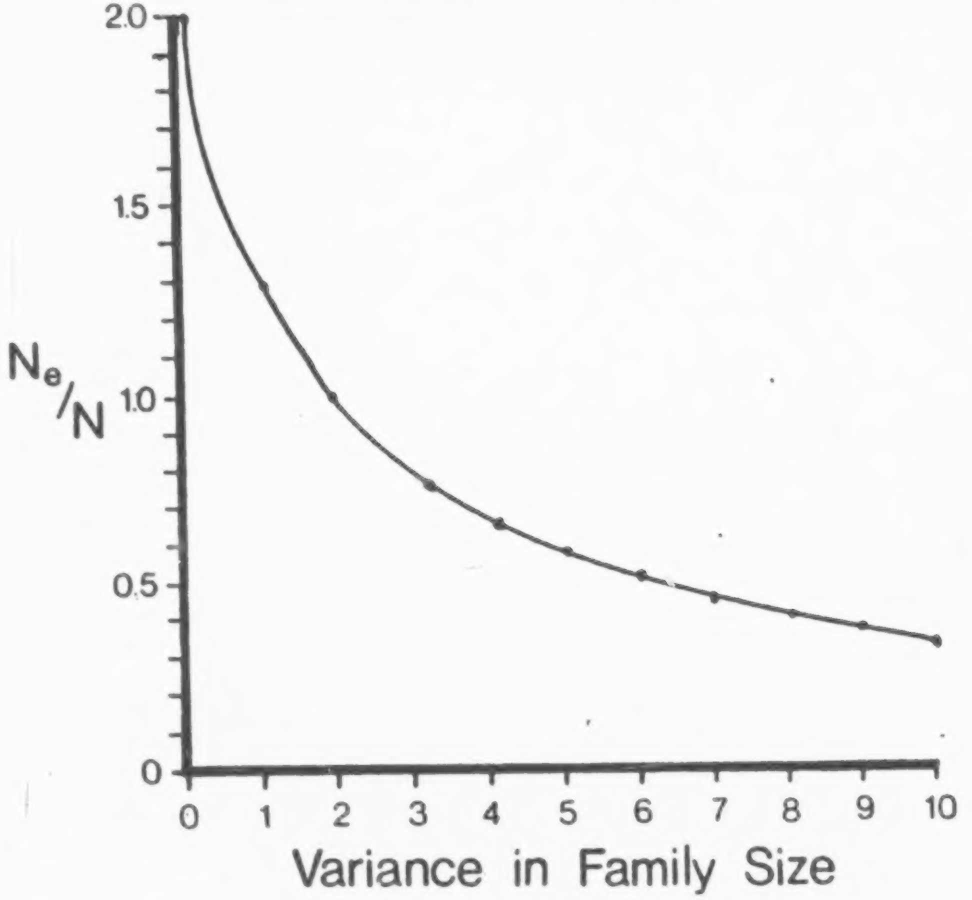


FIGURE 4.

Effective Population Size and Generation Times
for maintaining 90% of Genetic Diversity for 200 years.

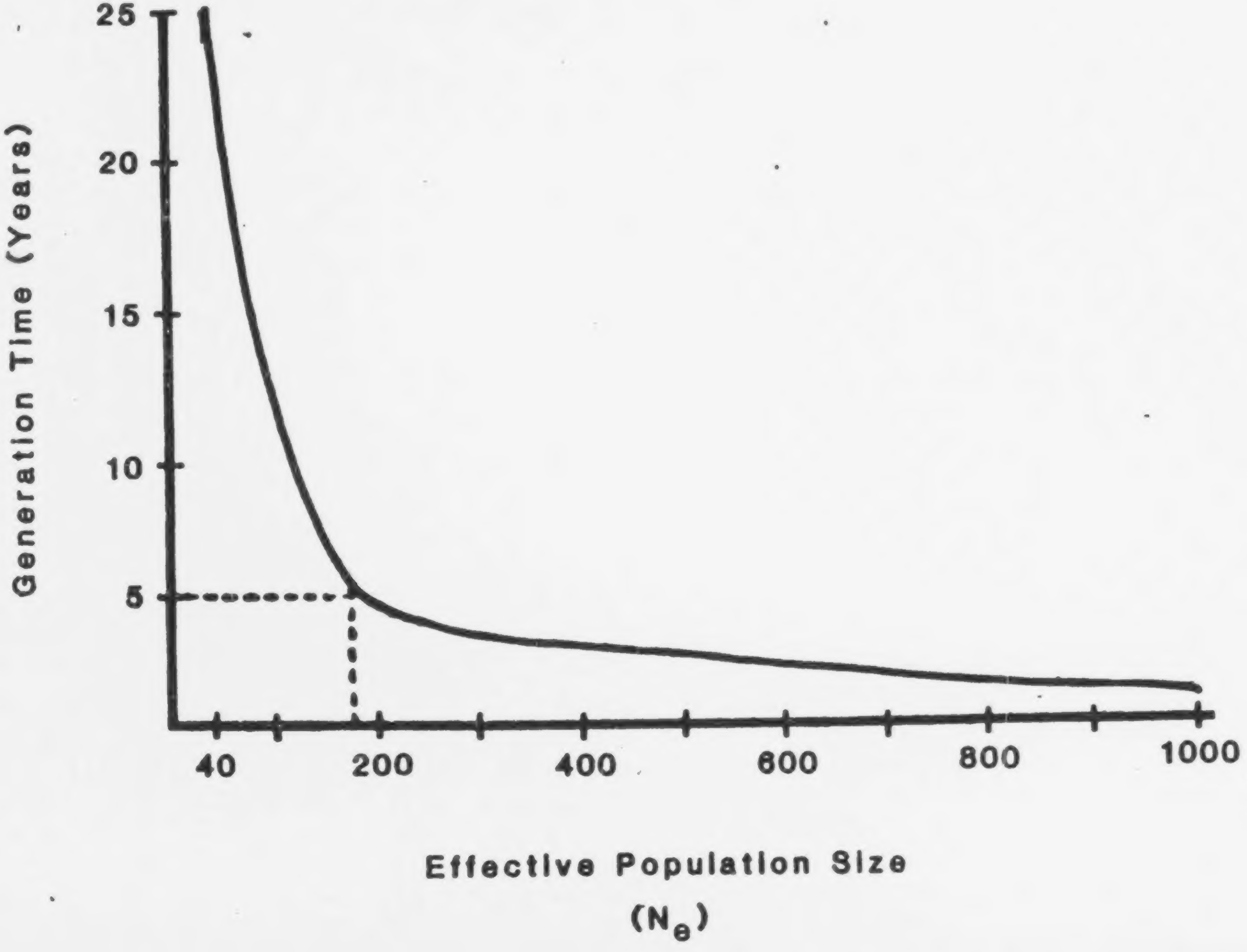
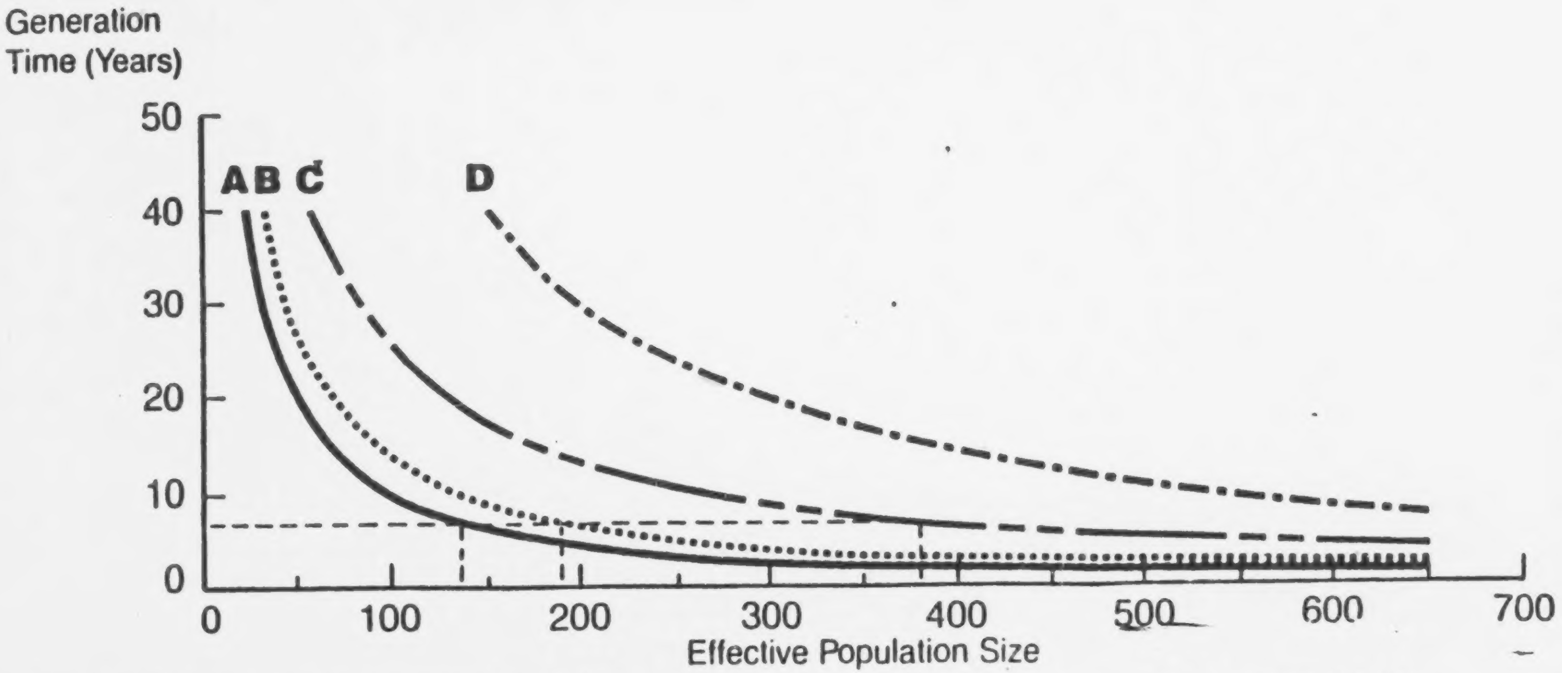


FIGURE 5.

Population size, founder size, generation time and preservation of diversity



Relationship between effective population size, generation length and founder group size required to maintain 90% of original genetic diversity for 200 years.

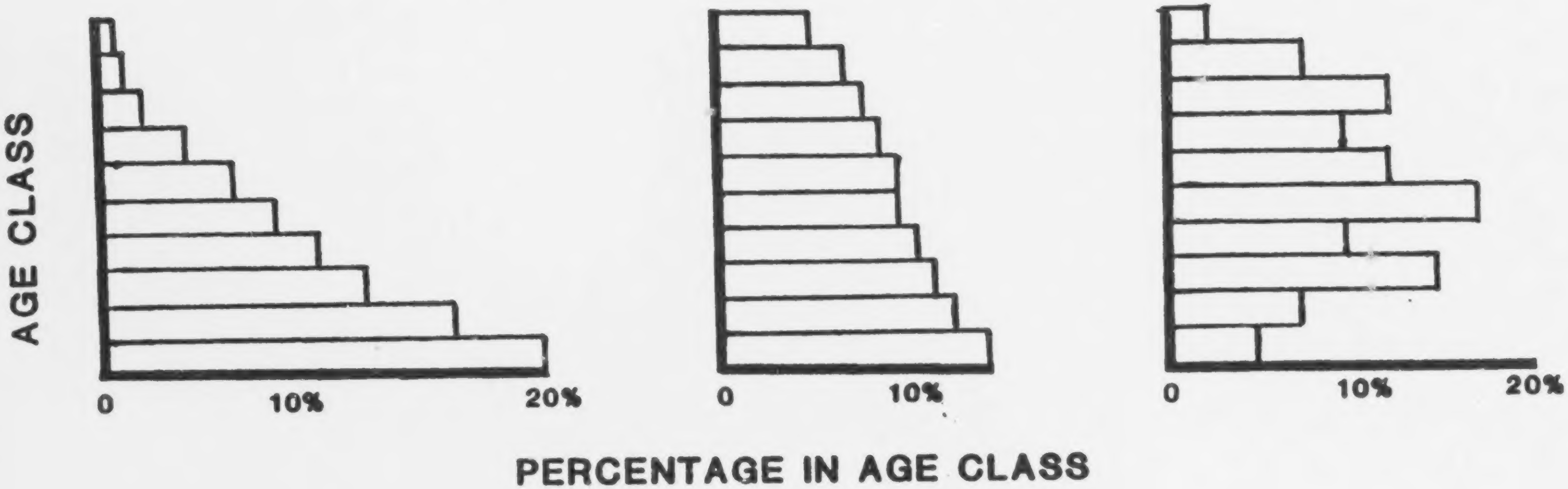
A—No founder effect **B**—20 founders **C**—8 founders **D**—6 founders

Assuming immediate expansion of founder groups with no loss of diversity (modified after graphics prepared by M. Soulé and J. Ballou).

(From Conway 1985)

FIGURE 6.

GENERAL PATTERNS OF AGE DISTRIBUTIONS

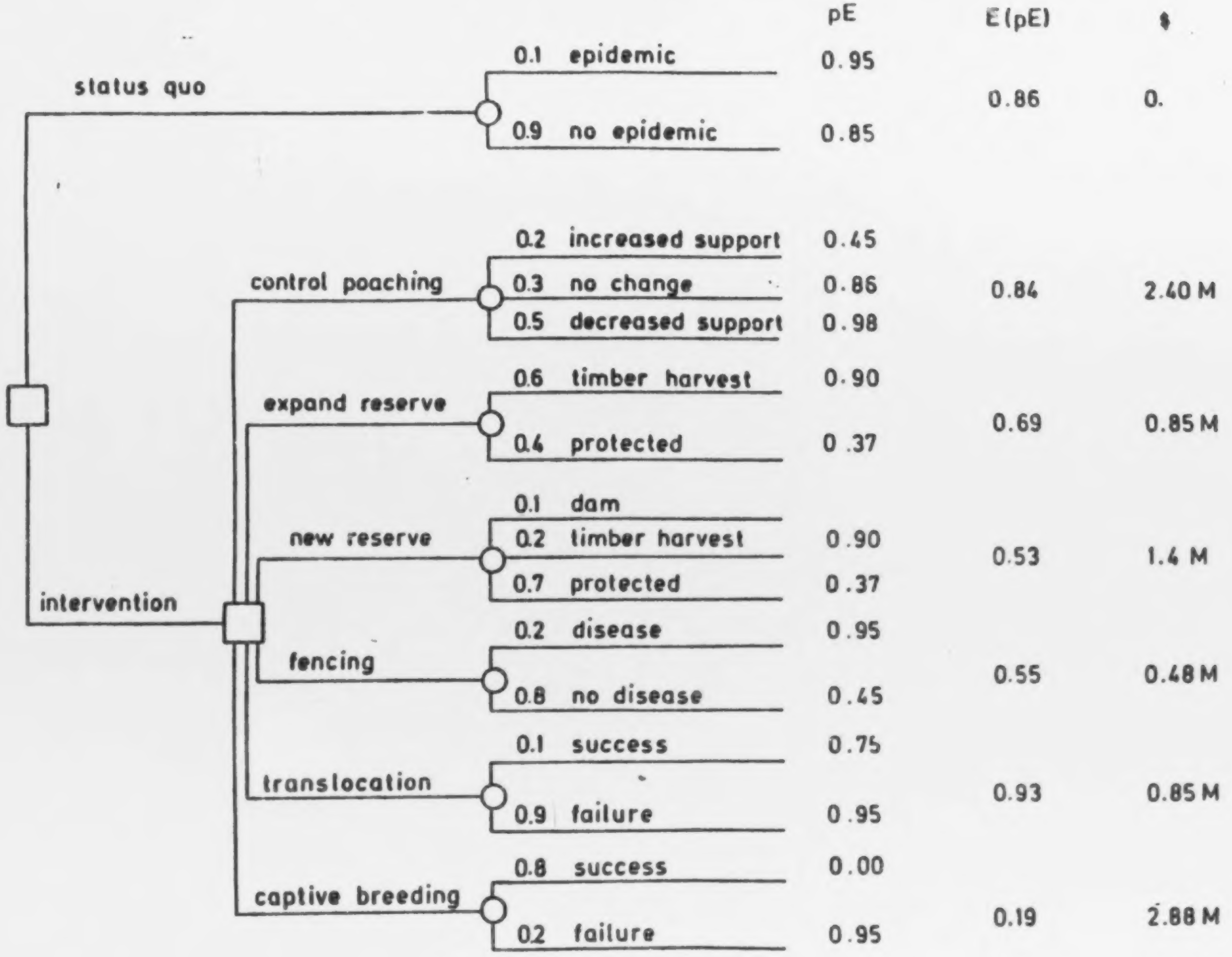


STABLE AGE DISTRIBUTION
POPULATION RAPIDLY GROWING

STABLE AGE DISTRIBUTION
POPULATION CONSTANT
IN SIZE

UNSTABLE AGE DISTRIBUTION

FIGURE 7. DECISION ANALYSIS FOR SUMATRAN RHINO


pE
E(pE)
$
status quo
0.1 epidemic
0.95
0.86
0.
0.9 no epidemic
0.85
intervention
control poaching
0.2 increased support
0.45
0.3 no change
0.86
0.84
2.40 M
0.5 decreased support
0.98
expand reserve
0.6 timber harvest
0.90
0.69
0.85 M
0.4 protected
0.37
new reserve
0.1 dam
0.2 timber harvest
0.90
0.53
1.4 M
0.7 protected
0.37
fencing
0.2 disease
0.95
0.55
0.48 M
0.8 no disease
0.45
translocation
0.1 success
0.75
0.93
0.85 M
0.9 failure
0.95
captive breeding
0.8 success
0.00
0.19
2.88 M
0.2 failure
0.95

pE = probability of extinction.
E(pE) = expected probability of extinction, i.e., the weighted average of the PE's indicated.
$ = cost of conservation action.
(From Maguire et al., 1986.)

Contract Paper No. 5

Management of Animal Disease Agents and Vectors Potentially Hazardous For Animal Germplasm Resources

Werner P. Heuschele
San Diego Zoo

October 1985

Office of Technology Assessment
U.S. Congress

This contract paper was prepared for the assessment of "Technologies to Maintain Biological Diversity." The views expressed are those of the author(s) and not necessarily those of OTA.

OCT 01 1985

MANAGEMENT OF ANIMAL DISEASE AGENTS AND VECTORS POTENTIALLY HAZARDOUS FOR ANIMAL GERMPLASM RESOURCES

* * * * * * *

Prepared for the Office of Technology Assessment,
Congress of the United States

Project: Technologies to Maintain Biological Diversity

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July 25, 1985

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ABSTRACT

Animal infectious disease agents and vectors can have serious impact upon animal health, reproduction and survivability. The successful preservation and maintenance of animal germplasm resources must involve the preventive management of hazardous animal disease agents and vectors. Regulatory controls and policies based upon the best available diagnostic technologies are especially important for the prevention of major epizootic animal diseases, particularly those foreign to the United States. Several newer technical methods (e.g., ELISA, immunofluorescence, molecular probes) provide the potential means for improved testing of animal germplasm for evidence of infection with important disease agents. Increased priorities and financial support for research and development leading to the routine application of improved diagnostic technologies for animal disease management prevention and control are urgently needed. Recent developments in embryo transfer and artificial insemination technologies offer the prospect that these methods may reduce the hazards of dissemination of major disease agents which in some cases are greater through movement of postnatal animals. When international movements of animals are necessary, foreign and U.S. pre-entry quarantine policies are essential for certain animal species to prevent the introduction of dangerous foreign animal diseases such as foot-and-mouth disease, African swine fever, rinderpest, and others. Use of the best diagnostic technologies to assure the freedom of animals and their germplasm from such disease agents before entry will provide orderly and safe circumstances for their importation.

Present U.S. quarantine procedures and facilities are adequate for currently regulated animals. The potential importation of foreign arthropod parasites capable of carrying and transmitting some foreign animal diseases, on animal species which are currently not subject to official regulatory controls, mandates that appropriate controls for such species must be developed without delay.

Improved testing technologies provide greater assurance that certain wild animal species (ruminants and swine) are free of disease agents of concern. The need for permanent post-entry quarantine (PPEQ) of such animals may therefore be unnecessary. This should be eliminated subsequent to a designated holding period under this status, e.g., one year. Reapplication of specified tests before such change of status would be appropriate.

Increased priorities and funding for research on the epizootiology (epidemiology), development of improved preventive methods, including state-of-the-art genetically engineered subunit vaccines, and basic research on the pathogenesis and immunology of major animal diseases of all categories of animals should be seriously considered and implemented. Without the necessary improved knowledge and technologies, many animal diseases will seriously jeopardize programs for the preservation of endangered animal germplasm resources, and will continue to impact and threaten rare species and food animal productivity worldwide.

Increased funding for research on germplasm cryopreservation, artificial insemination, in vitro fertilization, and embryo transfer for many endangered animal species is also urgently needed to increase captive propagation of such species for which the specter of extinction looms ominously.

I. INTRODUCTION

Increasing food animal productivity through selective breeding and genetic management has become an important practice in the livestock and poultry industries. In addition, selective breeding for disease resistance, which has many successful examples with plants, has become a matter of considerable interest for its potential application to food animal production.

The rapid decline in wild animal species, brought about largely by land use competition, overexploitation by hunting, trapping, and habitat destruction associated with rapid growth of the world human population, has brought many animals to the brink of extinction. Efforts by conservationists to establish self-sustaining captive populations of endangered animal species may be the only means of preserving a large number of highly imperiled species (Committee on Germplasm Resources, 1978).

It becomes readily apparent, therefore, that improvement of animal agriculture and endangered animal preservation will necessarily entail considerable domestic and international movement of animal germplasm in the form of semen, unfertilized ova, embryos, living tissues and cells, and postnatal animals. Accompanying such extensive animal germplasm transport comes the hazard of introduction and spread of dangerous animal disease agents and vectors, many of which may have enormous impact on animal viability, reproduction, and productivity. Indeed, certain animal diseases foreign to the United States, such as foot-and-mouth disease, African swine fever, or rinderpest could wreak havoc with costs in the billions of dollars, and drastic reductions in available food resources (McCauley, 1979a, b). Ironically, then, the transport of animal germplasm without appropriate safeguards may jeopardize the very conservation programs for which the transfers are required. Regulatory controls dealing with the management and protection of animal germplasm from important animal disease agents and vectors are therefore essential to programs for the preservation and maintenance of biologic diversity of animal germplasm resources. Various technologies are available to assure the freedom of animal germplasm from disease agents. With semen, ova and embryos, two broad approaches can be taken: (1) certification of the health of the donors and (2) certification of the germplasm itself as uninfected.

II. MAJOR ANIMAL DISEASES OF CONCERN

A myriad of infectious diseases caused by viruses, bacteria, fungi, protozoa, metazoan parasites, and arthropods, both foreign and indigenous to the United States exists (Gillespie and Timoney, 1981). While all may significantly impair animal health and productivity, certain of them must be considered of major importance because of their greater potential for high morbidity, mortality, and economic impact. Foreign animal diseases are of particular concern because of the high susceptibility of the U.S. animal populations to them, and because most of them produce especially severe disease manifestations with high morbidity and economic impact.

Table 1 lists the majority of diseases foreign to the U.S. and some of their characteristics. The most important of these will be discussed in greater detail in following sections of this paper. Certain animal diseases endemic or indigenous to the U.S. must also be considered as hazardous to animal germplasm resource management and conservation. These are listed in Table 2, and certain of them will also be discussed further in succeeding sections. Table 3 lists important diseases of non-ungulate animals including those of Canidae, Felidae, Procyonidae, Mustellidae, Viverridae, Ailuridae, Ursidae, Pinnipedia, Cetacea, Primates, avian and fish species, reptiles, amphibians and invertebrates. Table 4 lists important animal viruses by virus family.

Animal disease agents present in semen and interactive with embryos have been reviewed and listed by Stahlheim et al. (1979), Hare (1983) and McVicar (1984), and are therefore not reproduced here.

III. ASSESSMENT OF CURRENT KNOWLEDGE AND RESEARCH OF MOST IMPORTANT DISEASES

A. Foot-and-Mouth Disease

1. Epidemiology

Foot-and-mouth disease (FMD) is generally considered to be one of the most highly contagious animal diseases known. While mortality due to FMD is relatively low and primarily occurs only in young animals, its importance as the most threatening disease to the U.S. livestock industry is related to its very high morbidity and economic costs through severely impaired productivity (Callis, 1984).

Because of its essentially worldwide occurrence, the potential for its introduction into the U.S. through animal or animal product importation is a matter of serious concern. The broad spectrum of susceptible or potential carrier host species of FMD further aggravates its menace to the U.S. animal industry.

Transmission of FMD occurs by direct contact between infected and susceptible animals, and by indirect contact through airborne infectious aerosols, virus-contaminated feed, bedding, clothing, or other objects, and animal products, especially in the form of meat scraps in garbage. Arthropods are not considered important as vectors of FMD, but are capable of mechanically transporting the virus (Callis et al., 1982; Callis, 1984).

All Artiodactyla (even-toed, cloven-hoofed animals), both domestic and wild, are susceptible to FMD. Persistent latent infections have been demonstrated in cattle. Other species in which FMD susceptibility has been demonstrated include various rodents, hedgehogs, armadillos, elephants, and grizzly bears. Rare infections have occurred in humans. Experimental infection has been achieved in rabbits, chinchillas, young dogs, and cats. Domestic and wild ruminants and swine are the species with the greatest potential for disseminating FMD (Callis et al., 1982; Callis, 1984).

2. Relative Risk of Introduction by Various Germplasm Categories

a. Semen: Semen from FMD-infected bulls or boars may contain the virus, and when used in artificial insemination can transmit the infection (Callis, 1984; McVicar, 1984). Importation of semen from infected areas can, however, be safely accomplished by appropriate testing of aliquots of semen for the FMD virus and serologic testing of donor males for FMD antibody (Smith, 1978). Holding frozen semen until tests on donor males have been completed would have to be a part of such a program, as would holding the donor in quarantine for observation for a prescribed time (e.g., 10-14 days) after semen collection, to detect any animals in incubative stages at the time of semen collection. When these testing procedures are applied, the risk of FMD importation in semen is negligible.

b. Embryos: Embryos from FMD-infected cows can be rendered free of the causative virus by standard methods of embryo washing (i.e., at least ten washes), based on preliminary studies (McVicar et al., 1985). More extensive field trials to test the validity of these observations must be done before importation of embryos which have been washed, without additional testing can be recommended. Additional assurance of the freedom of embryos from virus can be provided by demonstrating that the donor dam and sire which produced the embryos were free of FMD at the time of the collection. Another approach which has been proposed is the direct testing of an aliquot sample (e.g., 10%) of embryos and their wash fluids collected from a single donor for the presence of virus. This, of course, would only be feasible if large numbers of embryos were obtainable. Combinations of some of the above methods might be used in some circumstances to increase the assurance of safety.

c. Postnatal Animals: Postnatal animals probably represent the riskiest means of transporting animal germplasm from the standpoint of disease transmission. Although current regulatory controls (Anonymous, 1985a) on importations of FMD susceptible animals (quarantine, serologic test for FMD antibodies, and sample testing for the presence of FMD virus in esophageal-pharyngeal scrapings) have very effectively prevented any introductions of FMD to the U.S., there is a risk, albeit remote, that a FMD test negative animal might still be a clinically inapparent carrier of FMD virus. Such cases have been demonstrated to occasionally occur in cattle (Callis, 1984; Gillespie and Timoney, 1981). Even so, the importation of exotic artiodactyla into the U.S. under existing regulatory controls has never resulted in an introduction of FMD into the U.S. (Goss, 1968).

B. African Swine Fever and Hog Cholera

1. Epidemiology

African swine fever (ASF) is the most serious of the swine diseases which threaten the U.S. swine industry (Hess, 1984; Callis et al., 1982). Hog cholera (HC) must be considered of equal importance, but is caused by an entirely different, unrelated virus. It induces clinical signs and pathology similar to ASF. All Suidae (domestic and wild pigs) are susceptible to and may serve as inapparent carriers of ASF and HC. ASF is usually severe in domestic pigs and European and Asian wild swine, but is usually carried as a clinically inapparent infection in African wild pigs such as the wart hog, bush pig, and giant forest hog (Hess, 1984; Callis et al., 1982; Heuschele, 1965; Gillespie and Timoney, 1981). Soft-shelled (Argasid) ticks of the genus *Ornithodoros* appear to be the natural hosts of ASF in Africa. A similar wildlife or arthropod reservoir for HC apparently does not occur; the reservoir being in inapparently infected domestic swine carriers. In Spain, the wild boar has become an important reservoir of ASF, as has also a species of argasid tick (*Ornithodoros erraticus*), which commonly inhabits piggeries (Hess, 1984).

Transmission of ASF occurs by the bites of infected *Ornithodoros* ticks. Both ASF and HC have been spread by the feeding of virus-containing pig meat scraps in garbage. The latter is the most common means by which ASF has been transported from one area to another. Once established on a swine-raising premise, ASF and HC spread readily by direct and indirect contact, including airborne transmission. Movement of swine which are subclinical carriers of ASF or HC has also served as a means of spreading these diseases from one swine raising area to another. This has been the principal means of HC spread (Carbrey, 1984).

2. Relative Risk of Introduction by Various Germplasm Categories

a. Semen: The virus of ASF has been demonstrated in the semen of ASF-infected boars (personal communication to J.W. McVicar by D.H. Schlater, cited by McVicar, 1984). Because of the wide distribution of both viruses within the body of an infected animal, it is logical to assume that semen from an infected animal is likely to contain the HC virus as well. Screening and testing procedures on semen and donor boars would provide the safeguards against importing virus-contaminated semen in a manner similar to that applied to FMD.

b. Embryos: Embryos contaminated with ASF virus have been found to retain residual ASF virus even after standard washing and trypsin treatment to remove the virus (Singh et al., 1984). Thus, on the basis of current knowledge and technologies, the importation of swine embryos from countries with ASF would create a significant risk of ASF introduction. This risk could be reduced by serologic testing of the donor dam and sire from which embryos are produced. The writer is not aware of any similar studies on HC virus-contaminated embryos; but since this virus is similar to BVD virus, it can be speculated that trypsin treatment and washing of embryos would render them free of HC virus. This obviously needs investigation.

c. Postnatal Animals: Importation of postnatal swine from countries where ASF and HC are present is currently not permitted because of the perceived risk that they might be carriers of ASF, HC, or several other important diseases of swine. Quarantine and application of currently available testing technologies to identify animals free of infection, as is done with ruminants for FMD, may provide the means for the safe importation of swine, both wild and domestic, from infected countries. Available evidence indicates that swine carriers of ASF or HC virus are seropositive for these respective agents (Hess, 1984).

C. Rinderpest and Pest of Small Ruminants

1. Epidemiology

Rinderpest, also known as cattle plague, is one of the most lethal diseases of cattle, sheep, goats, and various wild ruminants (Plowright, 1968; Maurer, 1984). Pest of small ruminants (PSR) is a serious rinderpest-like disease of sheep and goats (Hamdy, 1984). The causative virus is related to rinderpest virus, and is believed to have evolved from it. Swine have been experimentally infected with both viruses and could serve as disease vectors for a short period of time (Hamdy, 1984; Maurer, 1984).

Transmission of both diseases is by direct and indirect contact. The viruses do not multiply in arthropods, but probably could be transmitted mechanically by arthropods for a brief period of time.

In the case of both rinderpest and PSR, animals recovered from the infection apparently become immune and virus-free. Persistently infected carriers of rinderpest or PSR apparently do not occur. Further research on this point is, however, essential before this can be fully accepted as fact.

2. Relative Risk of Introduction by Various Germplasm Categories

a. Semen: It is likely that both rinderpest and PSR viruses would be present in semen from infected male animals. As with the previously discussed diseases, the testing of semen and donors for evidence of infection would still allow for importation of semen with relative safety.

b. Embryos: Although no studies have been reported on infection of embryos with these agents, it is assumed that washing, testing of aliquots of embryos and/or wash fluids for virus, and/or serologic testing of donor sire and dam would provide adequate safeguards for importing embryos from infected countries.

c. Postnatal Animals: Postnatal animals, after appropriate quarantine can, in the opinion of this writer, be safely imported with respect to rinderpest and PSR without specific testing. Animals completing quarantine for the time periods currently required (60 days abroad, 30 days in U.S. quarantine) would not constitute potential sources for introducing either disease. Even if they were in incubative stages of disease upon entry into quarantine, they would either manifest overt clinical disease while in quarantine and be sacrificed, or if only subclinically infected (as is often the case in sheep, goats, and some wild ruminant species), would develop immunity and be free of the infection within the quarantine period. Others feel that animals seropositive for rinderpest or PSR antibodies might be potential carriers. Therefore, research is urgently needed to clarify this question. The development of DNA probes to detect rinderpest gene segments *in vivo* in seropositive animals might be another approach to assure their freedom from virus.

D. Bluetongue, Epizootic Hemorrhagic Disease and Ibaraki

1. Epidemiology

Bluetongue (BT), epizootic hemorrhagic disease (EHD), and Ibaraki (IB) are insect-borne viral diseases of domestic and wild ruminants caused by related arboviruses (Osburn and Stott, 1984). Twenty-three serotypes of BT virus, seven serotypes of EHD virus, and one serotype of IB virus have been identified throughout the world. Clinical disease occurs with BT primarily in sheep and some wild ruminants. Although clinical BT occasionally occurs in cattle, in this host it is usually clinically inapparent. Deer and certain other wild ruminants are the principal clinical hosts for EHD; while Ibaraki disease, which is caused by a virus more closely related to EHD than BT virus, has only been recognized in cattle. EHD in cattle is usually subclinical.

Transmission of BT, EHD, and IB is primarily by hematophagous (blood-sucking) gnats of the genus Culicoides, which act as biological vectors. Ticks and some species of mosquitoes have also been shown capable of transmitting BT. Contact transmission between infected and susceptible animals does not occur.

All wild and domestic ruminants from countries or areas where the disease agents are present are considered potential carriers of BT and EHD viruses. At present, only cattle have been associated with Ibaraki infection, but because of its close relationship to EHD, all ruminants should be considered potentially susceptible to infection.

While these disease agents may produce serious overt disease and sometimes death, their greatest impact may lie in their more subtle adverse effects on reproductive performance, and on international trade of animals from infected countries. The presence of five serotypes of BT in the U.S. has severely limited the export of U.S. cattle, sheep, and goats. The introduction into the U.S. of any of the remaining 18 BT virus strains now foreign to this country, some of them more pathogenic than endemic U.S. strains, would further aggravate the problem this disease creates for the U.S. cattle and sheep industry, and would add to the disease hazards for native and captive exotic wild ruminant populations.

2. Relative Risk of Introduction by Various Germplasm Categories

a. Semen: Latent infections of BT and EHD viruses have been documented in cattle, sheep, and deer (Luedke, 1985). Semen from infected bulls and rams contains BT virus (Hare, 1983; Smith, 1978; Breckon et al., 1980; Bowen et al., 1983, 1985b; Luedke, 1985). Therefore, as with the diseases discussed previously, the testing of semen and donor males from countries where BT is present is essential prior to semen importation.

b. Embryos: Embryos when contaminated with BT virus may be rendered free of virus by proper washing (Bowen et al., 1985a).

c. Postnatal Animals: Importation of postnatal animals may be accompanied by a greater risk of introducing BT, than is importation of embryos. Immunotolerant seronegative cattle infected with BT virus have been reported (Luedke, 1985). This is believed to be a relatively rare occurrence; however, it represents some degree of hazard. Presently, the prevailing opinion is that animals seropositive for BT antibodies potentially harbor latent BT virus, and are thus possible sources of BT infection in areas where appropriate insect vectors are prevalent. On the other hand, other workers argue that animals seropositive for antibodies to a given strain of BT are immune to and free of infection with that strain (Osburn and Stott, 1984). The intracellular carriage of bluetongue virus in erythrocytes in infected animals with demonstrable serum antibodies has been documented. Such animals would therefore be potential carriers of bluetongue virus and sources for infection transmission in areas where appropriate strains of Culicoides vectors are available. This carrier status would, however, be only for the duration of the lifespan of their erythrocytes, which is about 100 to 120 days. This suggests that holding potential bluetongue carriers in gnat-proof quarantine for 120 days prior to release would obviate the hazards of importing bluetongue virus in infected animals. Thereafter, such animals would be immune to and free of the infecting virus strain (Gibbs, 1983, 1985). Further research is, however, needed before the controversy can be fully resolved.

The recent development of a genetic probe to detect BT virus infection in vivo and in vitro has recently been reported. The application of this method to seropositive animals and finding no BT genomic segments in such animals might be considered to provide assurance that they are free of the virus (Roy et al., 1985). This would decrease quarantine delays which now can be as long as two to three months while blood from seropositive animals is tested for the presence of BT virus.

The discussions of the foregoing diseases provide examples of criteria which may be applied to most other diseases with respect to importation or other transport of animal germplasm. As a general rule, the serologic testing of donor animals of semen or embryos, or of postnatal animals, can provide reasonable assurance that the subject germplasm is free of infection with a specific agent if the animals tested are seronegative for antibodies against that agent. With certain diseases in which carrier infections do not occur, the pre-entry quarantine currently required provides adequate safeguards against the importation of such diseases in postnatal animals.

In the case of diseases in which latent persistent infections are common, such as with most herpesviruses, ASF, or HC, the application of newer technologies such as molecular (DNA) probes to demonstrate the presence of nucleotide sequences representing the genome of the specific agents of concern in host cells, may provide assurances additional to those provided by serologic tests.

In situations where a representative sample (aliquot) of germplasm such as of semen or 10% of a multiple embryo collection can be obtained for direct testing for the presence of an infectious agent, this can provide additional safeguards against importation of disease agents in such materials. It can be argued, however, if tests on the donor dam and sire show them to be free of disease, the embryo can be assumed to be free also.

In some species, superovulation (multiple ova/embryos) has not yet been achieved. In such instances, testing the donor dam and sire for diseases of concern provides the best, if not only, assurance that the embryo is free of infection; provided the embryo collection and washing is done under circumstances which will preclude extraneous contamination with disease agents.

Several major problems confront strategies for conservation, propagation and disease control in non-ungulate animal species. First, regulatory controls to prevent the introduction and transfer of many hazardous diseases are non-existent for a majority of animals in this category. An example of this problem is illustrated by the recent occurrence of several episodes of feline infectious peritonitis in cheetahs (*Acinonyx jubatus*) in several zoos, with the resultant death of large numbers of this highly endangered species. In one instance, the disease followed the introduction of two new cheetahs from another zoo into a previously healthy collection. In the case of this disease, testing technologies for detecting carriers, and indeed specific diagnosis are not adequate. Funding support for research on diseases of livestock is insufficient; but, for non-livestock, is virtually non-existent.

Development of new technologies for artificial insemination, embryo transfer and *in vitro* fertilization of ova has moved forward rapidly for humans and some livestock species. Support for the development of similar knowledge and technologies for endangered species is urgently needed, though largely unavailable. The testing of animal germplasm of non-livestock species for hazardous disease agents is feasible, using some of the newer technologies to be described in the following section. However, while one can presume certain testing methods and/or treatment procedures may assure freedom of animal germplasm of such species from disease agents of concern by extrapolation of information derived for domestic livestock species; regulatory authorities, in some cases quite legitimately, may require research data which will unequivocally assure the validity of a given procedure for each exotic species of concern.

Even for domestic livestock, the development of knowledge that a particular treatment method which successfully renders embryos free of the infectious agent of one disease, may not necessarily be appropriate or acceptable to assure freedom of embryos from another disease. Much more research must therefore be done to provide appropriate data. This then also dictates an increase in available research support funding.

In considering the management of infectious disease problems of reptiles, amphibians, fish and invertebrates, one quickly recognizes a great paucity of knowledge even of what are the major causes of loss and impact. The list of recognized specific disease agents for these categories of animal life are quite short, due largely to the lack of support for, and hence research on them. Even for the known infectious diseases of such animals, laboratories with specific testing competence or interest are sorely lacking or non-existent.

The transfer of infectious diseases between wild and domestic animal populations is a matter of constant concern. Wildlife conservationists usually blame domestic livestock as the source of serious epidemic diseases which periodically dessimate large numbers of free-living wildlife. This is exemplified by the rinderpest epidemic in recent years in unvaccinated cattle in large areas of Africa. This soon spilled over into wildlife in some areas, resulting in the loss of large numbers of African buffalo and some other species (Estes, 1984).

Livestock producers, on the other hand, are quick to point the finger at wildlife as the source of many diseases occurring in their domestic stock. Indeed, malignant catarrhal fever carried by wildebeest in East Africa causes the loss of about 7% of Masai cattle by transfer of the causative virus from newborn wildebeest calves which act as healthy carrier-shedders of this disease agent. While these are but two examples of disease interchange between domestic and wild animals, suffice it to say that the transfer of disease between wild and domestic animal populations occurs in both directions with a number of disease agents. Prevention and control of major diseases is logistically much more feasible in domestic animals than in free-living wild animals. Widespread preventive vaccination of domestic stock against major disease problems, especially with the future generation of high-tech, genetically engineered, lower cost vaccines should markedly reduce the occurrence and impact of these diseases in livestock, and thus secondarily also in regionally cohabitant wildlife. In general, because of intensive production associated with close confinement of livestock, disease occurrence is usually greater in such animals than in wildlife.

IV. CURRENT TECHNOLOGIES FOR THE DIAGNOSIS OF DISEASE AGENTS

A. Applicable Methods (Gillespie and Timoney, 1981; Janetz et al., 1982)

1. Direct Demonstration of Agent in Tissues or Body Fluids

In the case of certain parasitic or bacterial infections, the causative agents may be abundant in blood cells, or other tissues, and may thus be readily demonstrated in smears, tissue impressions or sections. East coast fever (Theileriasis), malaria, babesiasis, anaplasmosis, heartwater and other rickettsial infections, and anthrax are examples of diseases which can be diagnosed in this manner. In the case of some viral infections, demonstration of viral inclusion bodies in stained tissue sections or impression smears may provide provisional diagnostic information (e.g., some herpesviruses, canine distemper, rabies, parvoviruses, poxviruses). Electron microscopic examination of ultrathin tissue sections, vesicular fluid from lesions, or feces may directly reveal the provisional identity of a viral agent based upon its characteristic morphology. Aggregation of viral particles by specific antisera visualized by electron microscopy provides a specific identification of the virus present and is the basis for diagnosis by immune electron microscopy. The latter has been particularly useful with enteric viral infections such as rotaviruses, coronaviruses, and parvoviruses.

Using direct or indirect immunofluorescence and enzyme immunoassay methods provides additional means to detect and demonstrate antigens of infectious agents in tissue sections and impressions and certain body fluids, particularly fecal extracts. Rabies, rotavirus, canine distemper, bovine viral diarrhea, African swine fever, and hog cholera are but a few examples of disease agents demonstrable by these methods.

The use of complementary DNA (c-DNA) probes derived from the cloned DNA or RNA of specific infectious agents has recently been shown to be a very promising method to demonstrate genome segments of certain infectious agents in tissue samples from infected animals. This method may prove to be of great value in identifying asymptomatic carriers of infectious agents which latently infect circulating white blood cells.

2. Culture and Isolation of Infectious Agents

Inoculation of cell cultures, chicken embryos, bacteriologic media, or experimental animals with samples such as blood, affected tissues, fecal extracts, exudates, nasal secretions, etc. are means for the direct isolation, cultivation, and identification of most infectious agents of concern. These methods, however, may frequently fail to demonstrate latent infections, particularly in situations where the agent is present in low titer (quantity), is sequestered in an inaccessible tissue, or is present in the form of a provirus with its genome integrated or otherwise associated with the host cellular DNA.

3. Demonstration of an Immunologic Response to Agents of Concern

As a general rule, immunologic tests, particularly serologic procedures, have been the preferred methods for demonstrating infection experience with a majority of infectious agents. One reason for this is that serologic procedures have traditionally been considered less costly and time-consuming than the methods for demonstrating the infectious agents described in the foregoing section. In addition, certain infection carriers have been difficult to identify by direct methods, for the reasons mentioned above.

a. Tests of Cell-mediated Immunity: The standard test for tuberculosis for many years has been the intradermal delayed hypersensitivity or tuberculin skin test. This test is one of several methods for demonstrating a T cell-mediated immune response. It has been successfully used to demonstrate infection with a number of other disease agents including the agent of glanders (*Pseudomonas mallei*), various fungal agents, *Mycobacterium paratuberculosis* (Johne's disease), etc.

The tuberculin skin test has for many years been the basis for the joint state-federal bovine tuberculosis eradication program. In areas other than the U.S., the mallein skin test has been the principal method to identify equines infected with glanders, a severe disease of horses, donkeys, and wild equine species which can also cause severe disease in humans.

Measuring a post-injection thermal response (fever) to antigens of *Mycobacterium paratuberculosis* of Johne's disease, injected intravenously has been a means of diagnosing this disease on a herd basis. This is yet another *in vivo* method for detecting cell-mediated immunity to some organisms. Culturing of feces of individual animals, or use of the ELISA method to detect serum antibodies are, however, superior methods to diagnose Johne's disease in individual animals.

Several laboratory methods are also available for detecting cell-mediated immunity to a variety of infectious agents. The lymphoblast transformation test has been the most studied of such methods. It has the drawback of requiring specialized equipment, live cultured lymphocytes from the blood of the test animal, and the use of radioactive isotopes; and thus has been used more as a research tool than as a standard diagnostic method.

b. *Serologic Tests for Antibodies*: A number of serologic tests for antibodies to infectious agents are available and have been used as standard methods for diagnosing infections and detecting potential carriers.

(1) *Complement fixation*. The complement fixation (CF) test is one of the oldest of serologic methods used to detect antibodies.

This test depends upon the binding (fixation) of complement, a group of substances present in normal blood serum, by antigen-antibody complexes. A positive reaction occurs when a test serum contains antibodies against antigens of the specific infectious agent with which it is being tested. Unfortunately, sera of some animal species or individual animals have substances or characteristics which bind (fix) complement in the absence of an antigen/antibody reaction. In these instances, a satisfactory negative test cannot readily be obtained by the standard complement fixation test method. The serum of donkeys, mules, asses, some horses, zebras and other wild equines, and cattle quite often causes this difficulty due to anticomplementary activity present in their serum. Newer testing technologies, such as will be described, should therefore be investigated as soon as possible with regard to their applicability to replace the CF test for diagnosing the above equine diseases, and other diseases where the CF test has at times been a problem.

The complement fixation test has been, and still is, a useful diagnostic method for the rapid specific diagnosis of vesicular diseases such as FMD, vesicular stomatitis, and swine vesicular disease. In this instance, it is used to detect specific viral antigens in vesicular fluid and lesion samples collected from clinical cases. However, enzyme immunoassay methods available today can be expected to be potentially better because of their greater sensitivity and ease of performance.

(2) Immunoprecipitation. Several variations of the immunoprecipitation test, particularly the agar gel immunodiffusion (AGID), the immunoelectro-osmophoresis or counter-immunoelectrophoresis (IEOP), and the radial immunodiffusion (RID) test methods have been useful for detecting antibodies to a number of infectious disease agents. The AGID test is the official test currently used to test for antibodies to bluetongue and EHD in cattle, sheep, goats, and wild ruminants, and for equine infectious anemia in equines. The IEOP test has been an important method used to test swine for ASF antibodies in herd outbreaks in the Iberian peninsula, the island of Hispaniola, and in Brazil in recent years. The AGID test has also been found suitable

for the demonstration of BVD, IBR, rinderpest, dourine, and FMD antibodies, among others. However, the immunoprecipitation test methods are less sensitive than certain other serologic test methods, such as virus neutralization, enzyme-labelled immunosorbent assay, and indirect immunofluorescence. The latter methods have, for many diseases, become the preferred serologic testing methods because of their greater sensitivity.

(3) Agglutination. Antibody-induced clumping or agglutination of particulate antigens such as bacteria is a useful means for demonstrating antibodies to certain bacteria, and for identifying bacteria isolated from clinical cases. The official diagnostic tests for brucellosis and leptospirosis are based upon bacterial agglutination by antibody-positive serum. Macroscopic, or the more sensitive microscopic agglutination methods are important in identifying specific serotypes of *Salmonella spp*. and serovars of *Leptospira interrogans*.

(4) Hemagglutination-inhibition. Certain viruses cause the clumping or agglutination of red blood cells (erythrocytes) of certain animal species. This phenomenon, called hemagglutination, occurs with influenza viruses, parainfluenza viruses, and other paramyxoviruses when reacted with erythrocytes of guinea pigs, fowl, or human type O blood. Several encephalitis viruses (e.g., VEE, WEE, EEE) will agglutinate goose erythrocytes, while many parvoviruses agglutinate pig erythrocytes. Newcastle disease virus regularly agglutinates fowl erythrocytes. Certain antibodies resulting from infection with these viruses will block or inhibit hemagglutination normally produced by these viruses. This then is the basis for the hemagglutination-inhibition test, which is routinely used in the serodiagnosis and virus

strain identification of influenza and other hemagglutinizing virus infections such as the equine encephalitides, parainfluenza, and Newcastle disease.

(5) Indirect hemagglutination-particle agglutination. When specially prepared erythrocytes (e.g., tanned or glutaraldehyde fixed) are coated with antigens derived from some infectious agents, such erythrocytes will clump (agglutinate) when exposed to solutions of serum containing antibodies against the infectious agent. A variation of this method to detect antibodies is found in the latex particle agglutination test in which latex particles are coated with infectious agent antigens instead of erythrocytes. These are then also visibly agglutinated in the presence of antibodies to the coating agent. These methods have been primarily used in research. However, commercial development of the latex particle agglutination method in the form of diagnostic kits is currently being pursued by at least one firm.

(6) Virus neutralization. The neutralization of virus infectivity by serum antibodies which result from infection or vaccination with a specific virus has been the basis for assessing infection experience and associated immune response for a majority of viral diseases. Several variations of the test method include: (1) the alpha method, wherein a constant dilution of serum is mixed with serial dilutions of virus; (2) the beta method, in which serial dilutions of test serum are reacted with a constant amount of virus; and (3) the virus plaque reduction-neutralization method, wherein several dilutions of test serum are mixed with a constant amount of virus, which is quantified by plaque assay. In each case the relative quantity (titer) of antibodies in the test serum is estimated by the amount of virus

infectivity reduction in relation to the concentration of the test serum.

With few exceptions, virus neutralization (VN) tests are highly specific and are applied not only to identify specific viruses or infection with them; but also, in the case of some viruses, to determine the particular serotype or subtype, strain or variant of a specific virus. This latter application is particularly useful with bluetongue, EHD, FMD, and vesicular stomatitis, among others.

In a few cases, crossreactions with the VN test occur with some related viruses. Occasionally this may lead to erroneous interpretations regarding the infection experience of a given animal. Rinderpest, canine distemper, measles and PSR, which are caused by closely related viruses of the genus, Morbillivirus, result in such crossreactive antibodies (Plowright, 1968). Similar crossreactivity is seen with the genus Pestivirus, consisting of hog cholera, bovine viral diarrhea and ovine border disease viruses (Carbrey, 1984).

For certain viruses, neutralizing antibodies are either not readily demonstrable in serum from infected animals, or do not occur. African swine fever virus, and some herpesviruses such as bovid herpesvirus type 4, fall in this category wherein the VN test is not applicable (Hess, 1984; Mohanty and Dutta, 1981).

An additional disadvantage of VN methods is that for some viruses there is considerable delay in obtaining results, depending upon the time required for the development of a visible effect produced by the test virus, such as cytopathology in inoculated cell cultures. In the case of bovine malignant catarrhal fever due to alcelaphine herpesvirus-1, final VN test results are not obtained until ten days after initial preparation of the test (Heuschele, 1984b). In general, the VN test requires 4-7 days for most viruses for completion (Gillespie and Timoney, 1981; Jawetz et al., 1982).

In spite of the cited disadvantages, the VN test has, until recently, been the preferred method for assessing the immune response to a majority of viral diseases.

(7) Indirect immunofluorescence. The indirect immunofluorescence (IIF) test method has recently gained popularity as a means of determining the presence of serum antibodies to a variety of infectious agents. Indeed, in the case of equine piroplasmosis (babesiasis), in which the current official test for antibody evidence of infection is the CF test, the IIF test method would be a better test since it is not confounded by the anticomplementary activity present in some equine sera (Kuttler et al., 1977; Kuttler, 1985).

An additional advantage of the IIF, and also of the enzyme-labelled immunosorbent assay (ELISA) test method to be next discussed, is rapidity of obtaining results. Both these tests can be completed within hours after obtaining test serum samples. The IIF test has been a valuable means of identifying ASF-infected swine, particularly with subclinical carriers or chronic infections.

A disadvantage of the IIF test method as conventionally used, is its occasional lack of specificity. Malignant catarrhal fever (MCF) is an example of a disease in which the IIF test reaction is positive not only with MCF virus, but also with some other herpesviruses (Heuschele, 1984a, b, c). In the case of MCF, the IIF test may be applied as a rapid screening method, to be followed up by the more specific VN test with positive reactor serum samples

(Heuschele, 1984b). Similar crossreactivity occurs with the IIF test method with some other infectious agents which are related to varying degrees, such as the Pestivirus and Morbillivirus group (Gillespie and Timoney, 1981).

(8) Enzyme-labelled immunosorbent assay. The enzyme-labelled (or linked) immunosorbent assay (ELISA) method is one of the more recently developed procedures for assessing specific antibodies resulting from infection with a variety of infectious agents. It has the advantage, as with the IIF test, of being rapid. In addition, by careful selection and use of purified subunit antigens unique to a given strain or serotype of an infectious agent, the ELISA test can be rendered highly specific. Its only drawback in the opinion of some critics is that in some instances it is too sensitive and may give false positive results. It can be argued, however, that more careful selection and purification of a limited amount of specific antigens, such as might be produced by recently developed DNA recombinant technologies, may obviate this problem and ultimately make ELISA the preferred serologic test method for a majority of infectious agents.

Measures to assure the freedom of embryos from infectious agents have been well-delineated (Hare and Singh, 1984).

V. APPLICATION OF VARIATIONS IN REGULATORY CONSTRAINTS FOR HANDLING ANIMAL GERMPLASM RESOURCES IN RELATION TO USES AND DEVELOPMENTAL STAGES

At the present time, import regulations contained in Title 9 CFR (Anonymous, 1985a), pertaining to exotic animals differ from those applicable to domestic animals. The essential difference is that domestic ruminants and swine are not allowed entry from countries where FMD, rinderpest, and certain other foreign animal diseases exist.

Exotic ruminants and swine from such areas are allowed for importation under permits by USDA approved zoos, subject to several stipulated conditions, procedures and criteria. These include: quarantine for 60 days in a USDA approved foreign quarantine facility; certification by animal health authorities in the country of embarkation that the animals have not been exposed to any infectious disease or manifested any evidence of such disease during quarantine. Upon satisfaction of these specifications the animals are then allowed U.S. entry only through the Port of New York. Upon entry the animals are placed in the USDA quarantine facility in New York for 30 days. During this period, blood samples and oesophageal-pharyngeal (O-P) scraping and fluid samples are collected and sent to the Plum Island Animal Disease Center for testing for evidence of FMD, rinderpest, and bluetongue infection. Upon satisfactory completion of tests and the 30 day post-entry quarantine, the animals are permitted to go to the approved zoo, where they enter into a status of permanent post-entry quarantine (PPEQ). In order to become an approved PPEQ facility, a zoo must meet criteria specified by the USDA which will assure that any potential foreign animal disease which might be carried by such an animal could not escape and be spread to domestic animals.

Importation of cell cultures and certain other animal products are regulated by the USDA (Anonymous, 1985a). In recent years industrial and research organizations have increased their interest in importing such items, particularly hybridoma cells for production of monoclonal antibodies, exotic animal tissue specimens for genetic study, and semen, ova and embryos for reproductive purposes. Many complaints have been voiced that import restrictions and testing requirements for such materials from foreign countries creates great hardships, costly delays and in some cases disadvantaged market positions for the intended importers. The contention is that requirements should be less restrictive for importation of germplasm for research and medical uses. In the opinion of the writer, removing or lessening certain import restrictions for such materials would increase the hazard of importation of foreign animal disease agents. Use of newer technologies (e.g., genetic probes, ELISA) to test samples of hybridoma or other cells for evidence of foreign animal disease agents, especially FMD, instead of the currently used animal inoculation tests should be considered to reduce the delays which are presently experienced before such materials are deemed safe for entry.

Exotic animals imported for exhibition in approved zoos should continue to be required to meet the quarantine and permit requirements, with exception of negative serologic tests for rinderpest, presently stipulated in Title 9 CFR. Serologic and O-P sample testing for FMD should also continue as stipulated. Serologic testing for rinderpest, as noted in the test methods section of this paper, may be unnecessary and on occasion has caused the unjustified exclusion of rare and endangered exotic animal species (e.g., giant eland, Jentink's duiker, zebra duiker) from entry into U.S. zoos. The present requirement for bluetongue antibody tests may be questionable. There is ample evidence that many exotic ruminants seropositive for exotic strains of bluetongue are present in U.S. zoos. Despite this, there is no evidence that the corresponding exotic bluetongue viruses exist in these animals or in domestic ruminants in the U.S. (House, 1982). This suggests that exotic ruminants seropositive for bluetongue antibodies are unlikely to be a means of introducing this virus, provided they are held in arthropod-proof quarantine for 120 days prior to entry/release as mentioned previously.

Newer testing technologies may be considered adequate means for providing assurances of the freedom of a domestic animal from certain exotic diseases. Their importation from uninfected premises of infected countries into the U.S. for breeding may be feasible upon further application and evaluation of these newer testing technologies.

Based upon preliminary studies, embryos of domestic and wild animals from infected countries, when properly treated, may provide the safest means of importing germplasm, subject to appropriate tests of donors previously discussed. Standard methods of washing embryos can provide further assurance of their freedom from infectious agents.

For certain highly endangered species, however, the technologies of embryo transfer may not be adequately developed in time to prevent the imminent or eventual extinction of that species. In such instance, importation of postnatal animals, using the best available applicable testing technologies, may be the only hope of preserving such valuable germplasm resources.

The technologies for increasing productivity of livestock through embryo transfer and related methods to assure freedom from certain diseases are well in hand for cattle. Development of similar technologies for many endangered species is lagging far behind.

It must therefore again be emphasized that funding for vigorous research on embryo transfer and related technologies for highly endangered animal species is urgently needed, lest certain species disappear, while we argue how they should be tested or regulated.

Ultimately it is likely that application of newer testing technologies should reduce the quarantine time needed for embryo and semen donors, and in some situations, for postnatal animals. However, for diseases with relatively long incubation times, shortening of required quarantine times for postnatal animals would not be advisable, based on current knowledge. As future research develops methods to detect infection in early incubative, presymptomatic or prodromal stages of disease, then shortened quarantine requirements may be realistically considered.

VI. COST/BENEFIT/RISK ASSESSMENT OF RELATIVELY PERMISSIVE VERSUS HIGHLY RESTRICTIVE REGULATORY POLICIES FOR GERMPLASM RESOURCES

Determining the preference of choice between a relatively permissive and a relatively restrictive regulatory policy for animal germplasm importation must consider some important tradeoffs.

A totally permissive policy without restrictions would be unthinkable in the light of projected estimated costs to the people of this nation which would result if FMD or ASF were introduced into the U.S. livestock population. It has been concluded in a study of the potential economic impact of FMD in the U.S. (McCauley and Sundquist, 1979b), that if FMD is introduced, as might readily occur with an unrestrictive import policy, resulting in a serious epidemic followed by an endemic situation with only voluntary control, the value of losses, calculated as consumer costs for animal products, is estimated at almost $12 billion for a 15-year period. This amount is the benefit derived from programs which prevent this undesirable outcome from occurring. This justifies present restrictive import policies by a benefit-cost ratio of 120:1 (McCauley and Sundquist, 1979b).

New technology leading to improved FMD vaccines and their essentially universal application could be considered as a possible tradeoff for allowing a more permissive import policy. However, in light of the logistical difficulties and costs of administering such a program, and the difficulty of predicting its adequacy in preventing disease, it is unlikely to provide a benefit-cost ratio even nearly approaching that of present restrictive policies.

Economically devastating avian diseases such as velogenic-viscerotropic Newcastle disease, and highly virulent avian influenza (formerly called fowl plague) would also make a totally permissive, unrestricted import policy for birds untenable (Anonymous, 1985b).

The long history of research with ASF with still no satisfactory preventive vaccine available argues strongly against a permissive import policy for swine. Because of the large size of the U.S. swine industry and large consumption and export marketings of pork and related products, the economic impact of ASF would quickly run into billions of dollars were it to gain entry into the U.S. (McCauley and Sundquist, 1979a). However, current testing technologies for ASF, HC, FMD and swine vesicular disease (SVD) may provide adequate means for assuring that swine or their embryos intended for importation from infected countries are free of these disease agents. A modification of the current exclusionary policy for swine requiring their quarantine and testing for the appropriate diseases (ASF, HC, SVD, FMD, pseudorabies, etc.) might therefore be viewed as an acceptable tradeoff. A similar policy for domestic ruminants also deserves consideration, in light of newer testing technologies which could be applied before entry.

The newer technologies for determining the infection status of animals, such as the enzyme immunoassay, immunofluorescence and molecular probe methods may well provide the means of safely importing animal germplasm in situations where such importations are currently prohibited.

VII. ASSESSMENT OF PRESENT QUARANTINE FACILITIES, POLICIES AND PROCEDURES

As indicated in foregoing sections of this paper, the application of some of the newer testing technologies for determining the infection status of animal germplasm for some important diseases is proceeding slowly. This is in large part due to a lack of adequate available funding support for the applied research and development needed to evaluate the surety of these methods for large scale standard useage. Increased federal funding is urgently needed to support both intra- and extra-mural research and development on the application and evaluation of new diagnostic techniques for certain key diseases. In many cases, e.g., equine diseases such as piroplasmosis, glanders and dourine, newer test methods would very likely eliminate some of the difficulties which occur with the current official tests for these diseases.

Once adequate research has been accomplished, which would prove the validity of certain new testing methods for assuring the infection-free status of animal germplasm intended for import or export; some of the current slower or less accurate official methods could be replaced by these newer procedures. Adoption of newer technologies which provide a high level of certainty of test results would then also permit modification of totally prohibitive import policies which currently prevail for some situations.

Current requirements for the pre-entry quarantine of certain animals and birds, should continue in force as necessary adjuncts to newer testing requirements. Animals in early incubative stages of a disease may test negative by diagnostic serologic or other methods, while appearing also clinically normal. The current pre-entry quarantine time requirements are sufficient to allow animals incubating an infectious disease to either develop clinical signs and/or a detectable immune response, other than the exception previously noted for bluetongue.

The permanent post-entry quarantine (PPEQ) requirement for imported wild ruminants and swine deserves reconsideration in the light of new diagnostic technologies. The present regulations stipulate that such animals may only be moved to another USDA approved (PPEQ) zoo. However, their offspring may be transferred to any location or facility. Since the imported animals had to be free of any evidence of infection with certain specified disease agents, or for that matter evidence of any infectious disease, it would be reasonable to assume, in the absence of any intervening evidence to the contrary, that they would still be free of such disease after a specified period in PPEQ status, say one year. Release of such animals from this restriction after one year in PPEQ status could be subject to satisfactory (negative) results for specified diagnostic tests before their release. Even this may not be necessary, since the longstanding policy allowing the unrestricted movement of offspring of PPEQ animals has never resulted in the dissemination of any foreign animal disease except one, malignant catarrhal fever (MCF). This herpesviral disease occurs as a subclinical and persistent infection in species of wildebeest, certain other species of African antelope, and in wild and domestic sheep and goat species. Transfer of this infection from mother to offspring occurs. Thus the progeny of imported PPEQ animals carrying MCF virus undoubtedly frequently were inapparently infected with this agent, and transported it to any locations to which they were moved. Current improved diagnostic methods for identifying MCF virus carriers can eliminate any continuance of this problem (Heuschele, 1984c). The recommendation has been made that all wildebeest intended for U.S. importation or interstate movement should be tested and serologically negative for MCF before allowing their entry into the U.S. or movement between premises within the U.S. Preliminary evidence indicates that latent sheep-goat-associated MCF viral infection may already be widespread in both domestic and wild sheep and goat populations in the U.S. and most other areas of the world (Heuschele, 1984a). Since the so-called sheep-associated form of MCF occurs only sporadically, requiring a seronegative MCF status for sheep and goat species for import, export or interstate movement has been considered logistically unsound. The situation in this instance is similar to that which prevails with bovine viral diarrhea-mucosal disease (BVD-MD), which is prevalent at a high level (60-80%) as an inapparent infection in the world cattle population (Gillespie and Timoney, 1981).

Restricting the movement of cattle on the basis of their BVD-MD serologic test status would virtually cripple the world cattle industry. In this case the economic impact of a restrictive policy with regard to BVD-MD would by far exceed the economic effects of the disease. A similar situation and policy thus is appropriate in regard to sheep-associated MCF.

Present U.S. quarantine facilities and related operating policies and procedures for imported domestic cattle, sheep, goats, swine, horses, poultry, pet birds, and related wild animal species falling in these categories appear to be for the most part satisfactory and adequate for the needs of current and projected import requirements. For reasons discussed previously, the extension of U.S. quarantine after foreign quarantine for bluetongue seropositive ruminants, from 30 to 60 days in arthropod-proof facilities, should be considered. While there have been requests to USDA to increase the number of quarantine facilities or enlarge some existent ones, the increased costs of such expanded facilities and associated operational expenses should be carefully considered in the context of increased demand for imports, especially of embryos, which is likely to occur. A precise estimate of the extent of embryo import numbers predicted for the future is not known to this writer. A significant increased importation of embryos and/or animals would certainly overtax current official laboratory testing capabilities. Increased facilities, staffing and budget to provide for increased import testing needs should therefore be considered.

There is also a need for more USDA-approved foreign quarantine facilities, particularly in Asia and Latin America. Importation of endangered ruminant, equine, swine and related wild animal species from these areas of the world is complicated and costly due to the absence of acceptable quarantine facilities in these areas of the world. Animals in these species categories from Asia or Latin America, must for the most part, be transported to the only available USDA-approved foreign quarantine facilities in Africa and Europe to meet the foreign pre-entry quarantine requirement. On occasions the animal health authorities of the countries in which the European quarantine facilities are located refuse the entry of certain animal species into these quarantine facilities. This seriously impedes importation of certain highly endangered animal species, greatly increases import costs, and compromises conservation programs for them.

In the past and at present, certain categories of wild animals have not been subject to any import inspection or quarantine requirements as it was believed that they did not represent any hazard from the standpoint of potential foreign animal disease introduction.

Recent, and a few past events have brought to light that this perspective can no longer be held. The direct importation of five black rhinoceroses from Natal in southern Africa to ranches in Texas during the past year led to the discovery that some of these animals harbored foreign tick species which serve as the principal intermediate host, reservoir and vector of heartwater, a serious foreign disease of sheep, goats, cattle and some other ruminants, caused by the rickettsial agent, Cowdria ruminantium (Wilson and Richard, 1984). Prompt tick control and eradication procedures by animal health officials succeeded in eliminating this hazard before it could become established. Similar past episodes of introduction of foreign ectoparasite vectors of disease on rhinoceros' and other non-regulated animal species have also occurred (Hayes, 1968).

New regulations requiring permits for, and the holding, inspection and treatment with suitable insecticide/acaricide preparations of certain previously unregulated animal species are currently being developed (Herrick, 1985). Animal species which have been subject to USDA regulatory controls have not created a problem in this respect as ectoparasite inspections and treatments are a part of the routine quarantine procedures for these species (Anonymous, 1985a).

Species under consideration for regulatory control for foreign ectoparasites include rhinoceros, elephant, hippopotamus and wild carnivores.

VIII. CONCLUSION

Although detailed discussion of the management of animal disease agents and vectors in this paper was primarily limited to some of the major diseases of domestic and wild hoofed animals, due to space limitations; programs for the management and preservation of animal germplasm must similarly consider major diseases of primates, carnivores, poultry, wild birds, reptiles, amphibians, fish and invertebrate species. Except for primates, which are subject to regulation for the prevention of certain diseases by the Department of Health and Human Services, and dogs regulated by USDA, DHHS, and state and local authorities for rabies control, species other than ruminants, swine, equines, poultry and wild birds are not subject to any infectious disease-related regulatory controls. Management and control of serious diseases which impact upon presently unregulated species must be considered in the overall program to maintain biological diversity.

The need for increased research, with appropriate funding, to evaluate the applicability of new diagnostic technologies for major animal diseases is imperative. In addition, support for research on epidemiology, improved preventive measures, including state-of-the-art genetically engineered subunit vaccines, and control programs for major diseases of regulated and non-regulated species, need increased funding.

If certain endangered species are to be preserved and sustained, research not only on their diseases but on their reproductive physiology, genetics, and the techniques of germplasm cryopreservation, artificial insemination, *in vitro* fertilization, and embryo transfer must be accelerated and supported as well.

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X. TABLE 1: MAJOR INFECTIOUS DISEASES OF ANIMALS - FOREIGN

Disease	Etiology (cause)	Usual Modes of Transmission	Incubation Period	Susceptible Natural Hosts	Subclinical Carriers Reservoir Hosts
African Horse Sickness	Orbivirus (9 serotypes)	Gnats: *Culicoides spp.*	3 days to 4 weeks	Equidae - horses, mules, asses; dogs	Zebras, elephants (?)
African Swine Fever	Iridovirus	Ticks: *Ornithodoros spp.*; contact with ingestion of contaminated garbage	5 - 15 days	Suidae - domestic and wild pigs	*Ornithodoros spp.* ticks; African wild pigs - warthog, bushpig, forest hog
Akabane	Bunyavirus	Mosquitoes and gnats	1 - 6 days	Cattle, sheep, goats	Not known
Bluetongue	Orbivirus (23 serotypes; 18 foreign to U.S.	Gnats: *Culicoides spp.*	7 - 10 days	Ruminants: cattle, sheep, goats, deer, antelope, etc.	Ruminants, gnats
Babesiosis					
Bovine	*Babesia* (6+ species)	Ticks: *Boophilus spp.*	2 - 3 weeks	Cattle, buffalo	Cattle, buffalo, ticks
Equine	*Babesia caballi*, *B. equi*	Ticks: *Dermacentor spp.*	2 - 3 weeks	Equines: horses, mules, asses, zebra, etc.	Equidae: horses, mules, asses, zebra, etc., ticks
Sheep, goats	*Babesia motasi*, *B. ovis*	Ticks: *Haemaphysalis spp.*, *Rhipicephalus spp.*	2 - 3 weeks	Cattle, buffalo; Equines - horses mules, asses, zebra, etc.; sheep, goats	Cattle, buffalo, ticks Equidae - horses, mules, asses, zebra, etc., ticks Sheep, goats, ticks
Swine	*Babesia trautmanni*	Ticks: *Boophilus*, *Hyalomma*, *Rhipicephalus spp.*	2 - 3 weeks	Swine	Swine, ticks

X. TABLE 1: CONTINUED

Disease	Etiology (cause)	Usual Modes of Transmission	Incubation Period	Susceptible Natural Hosts	Subclinical Carriers Reservoir Hosts
Bovine Ephemeral Fever	Rhabdovirus	Biting insects: midges, etc.	2 - 9 days	Cattle, water buffalo	Arthropods
Contagious Bovine Pleuropneumonia	*Mycoplasma mycoides mycoides*	Inhalation of infectious aerosols	3 - 6 months	Cattle, buffalo	Cattle, buffalo
Contagious Caprine Pleuropneumonia	*Mycoplasma mycoides caprae*	Isolation of infectious aerosols	6 days to 4 weeks	Goats	Goats
Dourine	*Trypanosoma equiperdum*	Sexual (venereal)	1 week to months	Equidae	Equidae
East Coast Fever	*Theileria parva*, other *Theileria spp*.	Ticks: *Rhipicephalus appendiculatus*, *Rhipicephalus spp*. *Hyalomma spp*.	8 - 12 days	Cattle, buffalo	African buffalo, ticks
Foot-and-Mouth Disease	*Aphthovirus* (7 serotypes)	Contact, aerosols, infected animal products, contaminated objects	2 - 7 days	All Artiodactyla - even-toed cloven-hoofed animals, hedgehogs, rodents, elephants, armadillos, grizzly bears, humans (rare)	Cattle, other artiodactyls
Fowl Plague - (Avian Influenza)	Influenza virus Type A, Subtype H7	Contact with secretions from infected birds	3 - 7 days	Most avian species, chickens, turkeys	Most avian species

X. TABLE 1: CONTINUED

Disease	Etiology (cause)	Usual Modes of Transmission	Incubation Period	Susceptible Natural Hosts	Subclinical Carriers Reservoir Hosts
Glanders	*Pseudomonas mallei*	Contact, ingestion of contaminated food and water	2 days to 2 months	Equidae: horses, mules asses, humans	Equidae
Heartwater	*Cowdria ruminantium*	Ticks: *Amblyomma hebraeum*, *Amblyomma spp*.	7 - 28 days	Cattle, sheep, goats buffalo	Cattle, sheep, goats, buffalo, African antelope
Hog Cholera	*Pestivirus*	Contact, contaminated garbage, water, objects	5 - 10 days	Suidae: swine	Suidae: swine
Lumpy Skin Disease	*Capripoxvirus*	Direct and indirect contact, flies	2 - 5 weeks	Cattle, buffalo	Cattle, buffalo
Malignant Catarrhal Fever (African)	Alcelaphine herpesvirus	Contact, aerosols, contaminated food and water	9 - 200 days	All ruminants: cattle, buffalo, deer, antelope	Alcelaphine antelope: wildebeest, hartebeest, topi; sheep, goats
Pest of Small Ruminants (Kata)	*Morbillivirus*	Direct and indirect contact	4 - 5 days	Sheep, goats	None known
Rift Valley Fever	Bunyavirus - *Phlebovirus*	Mosquitoes, occasionally contact	12 hours to 3 days	Cattle, sheep, goats, camels, buffalo, humans	None known, mosquitoes (?)

X. TABLE 1: CONTINUED

Disease	Etiology (cause)	Usual Modes of Transmission	Incubation Period	Susceptible Natural Hosts	Subclinical Carriers Reservoir Hosts
Rinderpest	*Morbillivirus*	Direct and indirect contact	3 - 9 days	All ruminants: cattle, sheep, goats, antelope, buffalo, swine	Swine - brief subclinical carriers
Sheep and Goat Pox	*Capripoxvirus*	Direct and indirect contact, flies	2 - 21 days	Sheep, goats	None known
Swine Vesicular Disease	Enterovirus	Direct and indirect contact, garbage	2 - 7 days	Swine, humans, sheep (?)	Swine
Trypanosomiasis	*Trypanosoma congolense* *T. brucei*, *T. simiae*, *T. vivax*, *T. evansi*	Tse-tse flies: *Glossina spp.*	4 - 40 days	Cattle, sheep, goats, pigs, horses, camels, dogs, cats, monkeys	Wild ruminants, wild equines, lions, leopards, wild pigs
Velogenic Viscerotropic Newcastle Disease	Paramyxovirus	Direct and indirect contact	4 - 14 days	All avian species, humans	Avian species
Venezuelan Equine Encephalomyelitis	Alphavirus	Mosquitoes	12 hours to 5 days	Equidae, humans	Rodents
Vesicular Exanthema of Swine	Calicivirus (13+ serotypes)	Contact, biting lice, contaminated garbage	18 - 72 hours	Swine	Marine fish, marine mammals

XI. TABLE 2: MAJOR INFECTIOUS DISEASES OF HOOFED ANIMALS - ENDEMIC IN USA

Disease	Etiology (cause)	Usual Modes of Transmission	Incubation Period	Susceptible Natural Hosts	Subclinical Carriers Reservoir Hosts
Anaplasmosis	Anaplasma marginale	Ticks, mosquitoes	20 - 40 days	Cattle, wild ruminants, deer, antelope, buffalo, bison	Domestic and wild ruminants
Bovine Viral Diarrhea	Pestivirus	Direct and indirect contact	4 - 21 days	Cattle, sheep, goats, wild ruminants	Cattle, sheep, goats, wild ruminants (?)
Brucellosis	Brucella abortus B. suis, B. melitensis, B. canis	Direct and indirect contact	2 weeks to 3 months	Cattle, wild ruminants, sheep, swine, goat, horse, wood rat, human	Cattle, reindeer, elk, etc., swine, goats, dogs, wood rats
Chlamydiosis	Chlamydia psittaci	Direct and indirect contact, respiratory, fecal, venereal	5 - 9 days	Poultry, wild birds, sheep, goats, cattle, wild mammals, humans	Psittacine and other wild birds, poultry, cattle, sheep, goats
Colibacillosis	Escherichia coli (enterotoxigenic strains)	Direct and indirect contact, fecal	12 - 18 hours	Cattle, swine, sheep, poultry, horses, dogs, wild ruminants, humans	Most mammals, poultry
Enzootic Pneumonia of Swine	Mycoplasma hyopneumoniae	Direct and indirect contact, respiratory	8 - 14 days	Swine	Swine

XI. TABLE 2: CONTINUED

Disease	Etiology (cause)	Usual Modes of Transmission	Incubation Period	Susceptible Natural Hosts	Subclinical Carriers Reservoir Hosts
Equine Infectious Anemia	Retrovirus	Hematophagous flies, mosquitoes	12 - 90 days	Equidae	Equidae
Equine Viral Abortion and Rhinopneumonitis	Equine herpesvirus-1 and -2	Direct and indirect contact, respiratory	Respiratory: 1-4 days Abortion: 3 weeks to 4 months	Equidae	Equidae
Equine Viral Arteritis	RNA virus (togavirus ?)	Inhalation	3 - 5 days	Equidae	Equidae
Equine Encephalomyelitis (Western, Eastern)	Alphavirus	Mosquitoes	1 - 5 days	Equidae, humans	Nestling birds, mosquitoes
Gastroenteritis (viral)	Rotaviruses Coronaviruses Parvoviruses	Direct and indirect contact, fecal	18 - 96 hours	Cattle, swine, horses, dogs, wild ruminants, cats, humans	Same as natural hosts
Infectious Bovine Rhinotracheitis	Bovine herpesvirus-1	Direct and indirect contact, respiratory, genital	3-5 days, respiratory, genital; 8 days to 4 months, abortion	Cattle	Cattle
Leptospirosis	*Leptospira interrogans* (150 serotypes)	Direct and indirect contact, ingestion	4 - 10 days	Cattle, swine, dogs, pinnipeds	Rats, dogs, swine, cattle, wild mammals

XI. TABLE 2: CONTINUED

Disease	Etiology (cause)	Usual Modes of Transmission	Incubation Period	Susceptible Natural Hosts	Subclinical Carriers Reservoir Hosts
Listeriosis	*Listeria monocytogens*	Ingestion	19-22 days	Cattle, sheep, humans	None, environmental
Ovine Progressive Pneumonia (also called Maedi)	Retrovirus (lentivirus)	Inhalation, ingestion, contact	Several months to years	Sheep, goats	Sheep, goats
Paratuberculosis (Johne's disease)	*Mycobacterium paratuberculosis*	Ingestion of infectious feces or contaminated food and water	Several weeks to months	Cattle, wild ruminants	Same
Pasteurellosis	*Pasterella haemolytica*, *P. multocida*	Direct and indirect contact, respiratory	Variable, 3 - 14 days	Cattle, sheep, rabbits, other ruminants	Same
Pulmonary Adenomatosis	Herpesvirus	Inhalation, ingestion, contact	Several months to years	Sheep	Sheep
Pseudorabies	Porcine herpesvirus-1	Direct and indirect contact with infectious nasal secretions of swine	2 - 6 days	Swine, cattle, sheep, goats, carnivores, rodents	Swine

XI. TABLE 2: CONTINUED

Disease	Etiology (cause)	Usual Modes of Transmission	Incubation Period	Susceptible Natural Hosts	Subclinical Carriers Reservoir Hosts
Rabies	Rhabdovirus Lissavirus	Bite from rabid animal, rarely inhalation	Several weeks to several months	Most mammals, especially carnivores, cattle, horses, humans	Bats, skunks, foxes, and certain other wild mammals - all become clinically affected
Scrapie	Viroid (Prion)	Contact through broken skin lesions	1 - 4 years	Sheep, goats	Sheep
Swine Dysentery	Treponema hyodysenteriae	Ingestion of infectious feces	10 - 14 days	Swine	Swine
Salmonellosis	Salmonella spp.	Direct and indirect contact, feces, contaminated food and water and objects	1 - 3 days	Any mammals, birds, reptiles	Mammals, birds, reptiles
Tuberculosis	Mycobacterium tuberculosis, bovis, avium	Direct and indirect contact, respiratory, oral	Several weeks to months	Cattle, swine, poultry, humans, horses, dogs, cats, wild mammals and birds	Same
Vesicular Stomatitis	Rhabdovirus Vesiculovirus (2 serotypes)	Direct and indirect contact, arthropods	2 - 4 days	Ruminants, swine, equines, humans	Bats, carnivores, rodents
Vibriosis (bovine)	Campylobacter fetus venerealis	Venereal	Variable	Cattle	Cattle
	C. fetus jejuni	Ingestion	Weeks to months	Sheep, cattle	Sheep, cattle

XII. TABLE 3: IMPORTANT DISEASES OF NON-UNGULATE ANIMAL SPECIES

A. CANIDAE

1. Canine distemper
2. Rabies
3. Canine infectious hepatitis
4. Canine parvovirus
5. Canine adenovirus-2
6. Canine parainfluenza
7. Canine herpesvirus
8. Canine brucellosis
9. Leptospirosis
10. Tuberculosis
11. Parasites

B. FELIDAE

1. Feline panleukopenia (parvovirus)
2. Feline rhinotracheitis
3. Feline leukemia
4. Feline infectious peritonitis (coronavirus)
5. Feline infectious hepatitis
6. Feline calicivirus
7. Feline pneumonitis (chlamydiosis)
8. Rabies
9. Parasites

C. MUSTELIDAE, PROCYONIDAE, VIVERRIDAE, AILURIDAE

1. Both canine and feline diseases are potentially hazardous, particularly canine distemper, canine and feline parvovirus
2. Parasites

D. URSIDAE

1. Canine adenovirus-1
2. Rabies
3. Leptospirosis
4. Parasites

E. PINNIPEDIA

1. Caliciviruses
2. Poxviruses
3. Leptospirosis
4. Parasites

F. CETACEA

1. Erysipelas
2. Parasites

G. PRIMATES

1. Poliomyelitis
2. Measles
3. Rubella
4. Monkey pox
5. Simian AIDS retrovirus
6. Salmonellosis
7. Shigellosis
8. Simian herpesvirus B and other primate herpesviruses
9. Marburg disease - rhabdovirus
10. Yellow fever
11. Respiratory and enteric viruses
12. Parasites, including malaria

H. AVIAN DISEASES

1. Newcastle disease
2. Avian influenza
3. Infectious bronchitis
4. Laryngotracheitis
5. Avian encephalomyelitis
6. Blackhead
7. Blue comb
8. Infectious bursal disease (Gumboro)
9. Fowl cholera
10. Coccidiosis
11. Pullorum, other salmonellosis agents (fowl typhoid, paratyphoid)
12. Fowl pox
13. Lymphoid leukosis (retrovirus)
14. Marek's disease
15. Mycoplasmosis
16. Avian adenoviruses
17. Chlamydiosis (ornithosis, psittacosis)
18. Pacheco's disease (psittacine herpesvirus)
19. Crane inclusion body disease (crane herpesvirus)
20. Raptor herpesvirus
21. Colibacillosis

I. FISH DISEASES

1. Parasitic diseases
2. Bacterial infections
   a. Aeromonas
   b. Pseudomonas
   c. Flexibacter

   - d. Vibrio
   - e. Corynebacterium
   - f. Mycobacteria
   - g. Nocardia
- 3. Virus diseases
   - a. infectious pancreatic necrosis (trout)
   - b. infectious hematopoietic necrosis virus (trout, salmon)
   - c. salmon herpesvirus
   - d. channel catfish herpesvirus
   - e. viral hemorrhagic septicemia (trout)

J. REPTILES

1. Paramyxoviruses
2. Herpesviruses
3. Bacterial infections
4. Parasites.

K. AMPHIBIANS

1. Lucke frog herpesvirus
2. Mycobacteria
3. Redleg (Aeromonas) and other bacterial infections
4. Parasites

L. INVERTEBRATES

1. Various viral, bacterial and parasitic diseases of lobster, crayfish, prawns (shrimp), oysters and clams.

TABLE 4: IMPORTANT VIRUS DISEASES OF ANIMALS

I. Viral Diseases - (By virus family)

A. Poxviridae

1. Genus *Avipoxvirus* - (avian pox) fowl, pigeons, turkeys
2. Genus *Orthopoxvirus* - cattle, buffalo, rabbits, camels, horses, humans
3. Genus *Suipoxvirus* - swine
4. Genus *Capripoxvirus* - sheep, goats
   - a. Includes lumpy skin disease of cattle
5. Genus *Leporipoxvirus* - rabbits, hares
   - a. Includes Shope fibroma
6. Genus *Parapoxvirus* - rabbits, hares
   - a. Includes contagious pustular dermatitis (sheep, goats, humans), bovine papular stomatitis (cattle), and pseudocowpox (cattle, humans)

B. Reoviridae

1. Genus *Reovirus*
   - a. *Reovirus* 1, 2, 3 (cattle, dogs, cats, horses, humans)
   - b. Avian *Reovirus* types (5) infectious tenosynovitis)
2. Genus *Rotavirus* - human, bovine, porcine, equine, ovine, canine - all associated with acute gastroenteritis, usually in neonates and young
3. Genus *Orbivirus*
   - a. African horsesickness - types 1-9, horses, asses, mules
   - b. Bluetongue - types 1-20, sheep, goats, cattle, wild ruminants
   - c. Epizootic hemorrhagic disease (Ibaraki disease), types 1-3, deer, wild ruminants, cattle
4. Unspecified genera of Reoviridae
   - a. Infectious bursal disease, young chickens and ratites (ostrich-like birds) (also called Gumboro disease)
   - b. Infectious pancreatic necrosis disease, salmonids

C. *Retroviridae*

1. Subfamily Lentivirinae

    a. Equine infectious anemia - horses
    b. Visna-maedi, sheep, cattle

2. Subfamily Oncovirinae

    a. Avian leukemia-sarcoma, chickens
    b. Feline leukemia-sarcoma, cats
    c. Bovine malignant lymphoma-leukosis, cattle
    d. Canine leukemia-sarcoma, dogs
    e. Murine leukemia, mice

D. *Herpesviridae*

1. Subfamily Alphaherpesvirinae

    a. Infectious bovine rhinotracheitis (IBR, BHV-1), cattle
    b. Bovine herpes mamillitis (BHM, BHV-2), cattle
    c. Equine rhinopneumonitis (equine viral abortion, EHV-1), equines
    d. Equine coital exanthema (EHV-3), equines
    e. Pseudorabies (PRV), cattle, sheep, goats, dogs, cats, rats and wild mammals, *swine* (reservoir host)
    f. Canine herpesvirus (CHV), canines
    g. Feline rhinotracheitis (FHV-1), felines
    h. Avian laryngotracheitis (LTV), chickens
    i. Pigeon herpesvirus, pigeons
    j. Raptor herpesvirus, hawks, owls, eagles
    k. Crane herpesvirus, cranes
    l. Duck plague (infectious duck enteritis), ducks, geese
    m. Simian herpesvirus B, macaque monkeys, man

2. Subfamily Betaherpesvirinae

    a. Atrophic rhinitis (inclusion body rhinitis), pigs
    b. Feline urolithiasis (FHV-2), felines

3. Subfamily Gammaherpesvirinae

    a. Malignant catarrhal fever (MCF, Alcelaphine HV-1, BHV-3) cattle, buffalo, wild ruminants, (*sheep*, *goats* + *alcelaphine antelope* reservoirs
    b. Pulmonary adenomatosis, sheep
    c. Marek's disease, chickens, turkeys, other fowl
    d. Rabbit herpesviruses - rabbits, hares
    e. Primate gammaherpesvirus, *H. saimiri* (squirrel monkey), *H. ateles* (Spider monkey) *H. tamarinus*, tamarins-marmosets

E. Picornaviridae

1. Genus Aphthovirus - foot-and-mouth disease (FMD), 7 types, over 60 subtypes, cattle and all other ruminants, swine, elephants, rodents, humans (rarely)
2. Genus Rhinovirus

    a. Bovine, types 1 and 2, cattle
    b. Equine, types 1, 2, 3, equines

3. Genus Enterovirus

    a. Teschen disease (PEV-1), swine
    b. Porcine enteroviruses, types 2-8, swine
    c. Swine vesicular disease (SVD), PEV-9, Coxsackie B-5), swine, humans
    d. Bovine enteroviruses, types 1-7, cattle
    e. Avian encephalomyelitis, chicks and pheasants
    f. Duck virus hepatitis, turkeys
    g. Turkey virus hepatitis, turkeys
    h. Human enterovirus, type 6, dogs (enteritis)
    i. Equine enterovirus, horses

F. Caliciviridae

1. Genus Calicivirus

    a. Vesicular exanthema of swine (VES), 13 serotypes, swine

        (1) San Miguel sealion virus (SMSV) closely related caliciviruses of marine animals, fish, seals, sealions, cetaceans

    b. Feline caliciviruses, several types, cats
    c. Bovine caliciviruses, cattle, other ruminants, enteritis
    d. Primate caliciviruses, apes

G. Togaviridae

1. Pestivirus

    a. Hog cholera (swine fever), (HCV), swine
    b. Bovine virus diarrhea-mucosal disease (BVD-MD, BVD), cattle sheep, goats, wild ruminants, swine
    c. Border disease of lambs (BDV, hairy shaker lambs), sheep

2. Rubivirus

    a. Equine arteritis, horses

3. Alphavirus
    a. Western equine encephalomyelitis (WEE)
    b. Eastern equine encephalomyelitis (EEE)
        (1) Natural hosts for WEE and EEE: birds, bird mites, mosquitoes, accidental hosts: horses, humans
    c. Venezuelan equine encephalomyelitis (VEE)
        (1) Natural hosts: rodents and other mammals, reptiles, birds?, mosquitoes, accidental hosts: horses, humans
4. Flavivirus
    a. St. Louis encephalitis (SLE), natural hosts: birds, bats, mosquitoes, accidental hosts: humans, horses.
    b. Louping ill, sheep
    c. Wesselsbron virus, sheep
    d. Turkey meningoencephalitis virus
    e. Japanese encephalitis - swine, wild birds, mosquitoes, horses, cattle, humans
5. California group encephalitis viruses
    a. La Crosse virus - prototype of group, rodents, mosquitoes, humans

H. Bunyaviridae
1. Rift valley fever, (RVF), sheep, goats, cattle, mosquitoes and other insects, humans

I. Myxoviridae
1. Myxovirus - Type A
    a. Avian influenzas -multiple subtypes (HN)
        (1) includes fowl plague
    b. Equine influenza, subtypes 1 & 2
    c. Swine influenza, 1 subtype, multiple variants

J. Paramyxoviridae
1. Morbillivirus
    a. Canine distemper (CDV), canines, procyonids, mustellids, viverrids
    b. Rinderpest (RDP) - cattle, all ruminants
    c. Pest of small ruminants (PSR), sheep, goats
    d. Measles, primates, humans

2. Paramyxovirus
    - a. Newcastle disease (NDV), fowl and wild birds
    - b. Parainfluenza-3 (PI-3), cattle, sheep, goats, wild ruminants, horses, humans
    - c. Canine parainfluenza (SV-5), dogs
    - d. Parainfluenza-1 (Sendai) swine, humans
    - e. Mumps, cats, dogs, humans

3. Pneumovirus
    - a. Respiratory syncytial virus (RSV)
        - (1) Bovine, cattle
        - (2) Human, primates

K. Coronaviridae

1. Infectious bronchitis (IBV), multiple serotypes, chickens
2. Transmissible gastroenteritis (TGE), 1 serotype, swine
3. Bovine coronavirus, 1 serotype, cattle
4. Hemagglutinating encephalitis (HEV), 1 serotype, swine
5. Canine coronavirus, 1 serotype, dogs
6. Feline coronavirus, multiple serotypes, cats
    - a. Feline infectious peritonitis (FIP)
    - b. Enteric
7. Equine coronavirus, horses, enteritis
8. Bluecomb, turkey TGE, 1 serotype

L. Rhabdoviridae

1. Rabies, all warm-blooded animals, especially canines, mustellids, procyonids, bats, humans
2. Vesicular stomatitis (VSV), horses, cattle, swine, other ruminants, humans
3. Bovine ephemeral fever, cattle

M. Parvoviridae

1. Feline panleukopenia (FPL, feline infectious enteritis), cats, mustellids, procyonids, viverrids, ailurids
2. Canine parvovirus (CPV), canines, procyonids
3. Raccoon parvovirus, procyonids, canines
4. Porcine parvovirus, swine (SMEDI virus)
5. Bovine parvovirus, cattle
6. Goose hepatitis virus, geese
7. Aleutian mink disease, mink

N. Papovaviridae

1. Rabbit papilloma virus
2. Bovine papilloma virus
3. Canine papilloma virus
4. Bovine papilloma virus
5. Sheep papilloma virus
6. Goat papilloma virus
7. Deer papilloma virus

O. Adenoviridae

1. Infectious canine hepatitis (ICH, canine adenovirus-1, fox encephalitis), canines
2. Canine adenovirus-2, canines
3. Bovine adenovirus, types 1-8, cattle, other ruminants
4. Porcine adenovirus, types 1-5, swine
5. Genus *Aviadenovirus*, several species affecting fowl, waterfowl

P. Iridoviridae

1. African swine fever (ASF), swine

Q. Arenaviridae

1. Lymphocytic choriomeningitis (LCM), mice, man, rodents, lagomorphs

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Constraints to International Exchange of Animal Germplasm

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Consultant

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Title

CONSTRAINTS TO INTERNATIONAL EXCHANGE OF ANIMAL GERMPLASM

by

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Congress of the United States
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September 1985

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CONSTRAINTS TO INTERNATIONAL EXCHANGE OF ANIMAL GERMPLASM

1) Constraints relating to introduction of germplasm into the U.S. are derived from existing regulations applicable to animals and animal products. Current legal and regulatory constraints to movement of animal germplasm into, through or from the United States are primarily related to disease transmission or potential transmission in animal populations. Acts of Congress and resulting regulations attempting to limit introduction of animal diseases to the U.S. have evolved from over 100 years of experience in assessing the effects of contagious animal diseases on: a) the efficiency of agricultural production and the value to U.S. agriculture of disease-free populations and b) on the eligibility of U.S. animals and animal products for international commerce.

2) Recent research efforts to improve viability of germplasm while being stored or transported implies the practicality of continuing and broadening the initial objectives. Similarly, techniques for the identification of potential pathogens and, perhaps of equal significance, the potential of having techniques that will assure germplasm free from potential pathogens, would warrant fundamental reevaluation of current safeguards oriented toward limiting disease transmission.

3) Past and existing international treaties and/or commissions relating to international movement of animals or animal products generally do not contain separate provisions for the movement of animal germplasm into the U.S. There have been a few exceptions covering the importation of bovine semen into the U.S. There are currently negotiations being carried out with other countries to conduct research that may provide information toward facilitating safe movement of animal germplasm.

4) Tariff related trade barriers concerning importation into the U.S. are the concern of the Department of the Treasury and the Department of Commerce, often acting in concert with the Department of Agriculture. They have not been applied to germplasm. Non-tariff related barriers are mainly those by USDA and are related to disease transmission, or by the Department of the Interior when they concern endangered or noxious species of animals.

5) The advantages of evolving safe procedures for the movement of germplasm to and from the U.S. are identified or implied throughout the Project. It is not expected however that there will be significant modification of legislative or agency policy on exchange of animal germplasm without new technological information. In view of the overriding advantages of a disease-free animal population, a commitment must be established to develop new technologies to assure the U.S. of safe exchange.

ANIMAL GERMPLASM MAINTENANCE AND USE

1. Legal and Regulatory Constraints to International Commerce

With a century of successful disease eradication and surveillance programs, the U.S. livestock industry has established an enviable reputation for its relative disease-free status in the world today. The evolutionary development of these concepts relative to social and economic changes are reflected in the current constraints on international traffic in animals and animal products which include germplasm.

In the 1870's, the US Livestock Industry grew to the extent that foreign markets were needed. Entrepreneurs shipped cattle to England for slaughter; carcasses were shipped under refrigeration; and pork was being canned for export. In the butcher shops of London and Dublin, U.S. beef and pork became extremely popular as it was considered better and was certainly cheaper.

However in 1878, both England and Germany banned the entry of U.S. cattle, sheep and meats on the grounds that they were infected by disease.

As a result of rising demand by the U.S. livestock industry for international markets on one hand, and the obvious discrimination against animals and animal products of U.S. origin due to the prevalence of disease, real or perceived, on the other, Congress, in 1884, passed an Act to establish the Bureau of Animal Industry. Its purpose was "to prevent and to facilitate the eradication efforts under way", and appropriate funds to purchase and destroy diseased and exposed animals.(1)

While England declared an embargo of beef imports from the U.S. due, they said, to the presence of contagious bovine pleuropneumonia (CBPP) in that country,it is interesting to compare the figure of $1.5 million required for the total eradication of CBPP, to that of $1 million per year lost through this embargo.

The U.S. was fortunate in the beginning. This was a new land free of the many devastating animal diseases that plagued Europe, Asia and Africa. Movement of human and animal populations to the new land took place by long sea voyages. The period of time involved for these voyages served as a quarantine period by concentrating humans and animals, providing diseases with the opportunity to become apparent. On arrival in the new land, affected individuals had usually either died and been disposed of, or they were in the process of recovering, or were immune.

Contagious bovine pleuro-pneumonia has however a protracted incubation period and gained access to the U.S.

through a diseased cow arriving in the Port of New York in 1843, interestingly aboard a British ship.

Foot-and-Mouth disease (FMD) is of major concern to the livestock producing countries in the World. The first outbreak in the U.S. was reported in 1870 and there have been several subsequent ones. Following the last minor outbreak in California in 1929, the U.S. Congress passed the Tariff Act of 1930. This provided an embargo against the importation of cattle, sheep, other domestic ruminants and swine or products from these animals from any country known to harbor FMD. Since the passage of this Tariff Act, the U.S. has not had an outbreak of FMD in its national herd. However its persistence as a hazard to the livestock industry has been apparent through subsequent outbreaks in both Canada and Mexico.

A recent study by the University of Minnesota indicates that the benefits to consumers for keeping FMD out of the U.S. (discounted at 8 percent per year), amounts to $11.65 billion for the period 1976-1990.(2) This estimate does not include consumer choice or export market benefits attributable to the avoidance of FMD. FMD-free countries could and presumably would, in the event of an epidemic, ban imports of U.S. livestock and livestock products. A severe series of outbreaks and a strict embargo for 12 months or longer would have a minimum annual impact of $870 million in lost markets, at 1980 values. To this would have to be added costs of reestablishing those markets and of losses in related industries such as transport and shipping.(4)

Present policies restricting the import of animals and animal products from FMD infected countries are justified by a benefit/cost ratio of 120:1.

Since 1970, the value of U.S. trade in livestock, meat and animal products has increased approximately fivefold. Since 1979 there has been a relatively constant level of animal related exports exceeding $3 billion per year in spite of the continued strength of the U.S. dollar and its deterrent influence on world trade.(3)

The major zoosanitary constraints levelled against international commerce in animals and animal products relate to FMD. The U.S. has been free from FMD since 1929 and the North American Continent since 1953. It is therefore not surprising that by far the bulk of animal related exports are to countries that are likewise free of FMD and anxious to maintain the advantages related thereto. It is not purely coincidental that these other countries of the world are those that have stable currencies.(3)

Estimates prepared by a National Research Council Subcommittee indicate that there would be a total benefit of $12 billion by keeping FMD out of the country. Cost to control a modest outbreak of FMD would be $54 million but other studies indicate that this figure might be at least ten times higher. The benefit of keeping African Swine Fever (ASF) out of the U.S. has been estimated at $5 million. The cost in 1981 dollars to eradicate a small ASF outbreak, similar to the 1976 New Jersey hog cholera experience, has been estimated at approximately $12 million. The eradication cost of a multi-state ASF outbreak has been estimated at more than $152 million. A repetition of Mexico's 1946-1954 FMD epidemic would cost $404 million to eradicate today. The U.S. is necessarily involved when that kind of disease nears its borders and it shares an interest with its neighboring countries in surveillance and control of diseases and disease vectors.(4)

Another factor relating to the presence and persistence of disease, and frequently not identified, is its effect on consumer prices. This has been clearly noted in the recent outbreak of avian influenza. Even though the losses to the affected producers were approximately $508 million, consumers have borne more than ten times that loss as a result of shortages which increased food protein costs during the 6 months period between November 1983 and April 1984.(5)

It is appropriate to emphasize the size of the industry at risk when considering the restrictive safeguards that have been put in place to maintain a disease-free livestock industry. Using U.S. census figures, the cattle herd of the U.S., both beef and dairy, have an on-farm value of appproximately $55 billion producing an annual income of about $25 billion. Thus, the cattle industry alone is equivalent to approximately an $80 billion resource to the U.S. economy.

In considering the average annual export earnings from animals and animal products in the vicinity of $3.5 billion, it is noted that bull semen, as a category, is equivalent to approximately $25 million. During previous years, export of embryos, almost exclusively bovine, was just under the $500,000 minimum limit for inclusion in the Foreign Agriculture Service (FAS) schedule of export commodities. However, during the current year it is anticipated to be in excess of that figure and will be recorded accordingly.

Current requirements for the importation of certain animals and poultry and their products are contained in Part 92 of Title 9 of the Code of Federal Regulations (9CFR92).

The appropriate authorities for the regulations described are cited in the introductory paragraph of Part 92. The bulk of the regulations are taken from 92 FR 5971 of June 13, 1963. In addition, Part 94 contains specific prohibitions resulting from the Tariff Act of 1930.

Sections of 9CFR92 describe general requirements for importation of animals based on health related concerns. The sections likewise designate ports of entry for various species and/or geographical origin. They also identify requirements to be met for ruminants, swine, horses, poultry, semen, pet birds, zoological species and for birds used in research.

While the Tariff Act of 1930 specifically prohibits the entry of ruminants and swine and products derived from them from countries infected with FMD and rinderpest, the new Harry S. Truman Animal Import Center (HSTAIC) at Fleming Key, Florida, dedicated in 1979, permits maximum security quarantine for imported cattle. They may be imported directly from countries infected with FMD into this quarantine facility which can accommodate up to 230 cattle for each of the three 90 day quarantine periods possible per year.

Space for accommodation at the HSTAIC is available by lottery or application for exclusive use. Authorization for assembling animals for entry at HSTAIC must be obtained in advance of provisions for moving the animals into an approved embarcation quarantine in the country from which they will be shipped to HSTAIC. Costs relative to assembling, testing and holding in the quarantine facilities both in the country of embarcation and in Fleming Key, Florida are borne by the shipper and include costs incurred by travel of USDA personnel, the utilities provided at the facilities, laboratory fees associated with determining the animals' freedom from disease plus an overhead fee. These facilities could also be used for the importation of animal germplasm.

Other sections of Part 92 contain special provisions applicable to the importation of animals and animal products from Canada, Mexico, Central America and the West Indies. These special provisions reflect the contiguity of the respective national herds, disease exposure, control strategies and patterns of commerce by the countries of North America. Continued emphasis is on the maintenance of FMD-free populations which remains a major concern among this group of nations.

Based on the recent development of laboratory techniques for the diagnosis of FMD and interpretations related to natural versus vaccine-induced antibodies in cattle, regulations were relaxed by an action effective November 2, 1984 by Veterinary Services-Animal Plant Health Inspection Service (VS-APHIS) of the USDA (9CFR Part 92.4). Even more recently, on January 14, 1985, proposed rules were published in the Federal Register dealing with the importation of certain animal embryos (9CFR Part 93). A public hearing was held in Kansas City on May 15, 1985, at which interested groups expressed their reaction to the proposed rules and made suggestions for modifications based on their specific interests. (6)

At the Annual Meeting of the International Office of Epizootics (OIE), held in Paris in May 1985, a major topic on the agenda dealt with "Diseases Transmissible by Semen and Embryo Transfer Techniques".(7)

Animal products in transit through the U.S. are subject to the same regulations as would be the case were they offered for entry into the country.

The meeting held by VS-APHIS of USDA in Kansas City in May 1985 as well as that held the same month in Paris by the OIE indicate the current awareness of the potential for the further development of international transportation of animal germplasm, and the ensuing mandate to establish satisfactory safeguards to avoid disease transmission. The statement made by the President of the U.S. Animal Health Association at the Meeting in Kansas City appears highly appropriate at this stage: "that the recommendation is, as VS-APHIS has done admirably, GO SLOW, on Part 93; be sure of our direction and develop a system of risk evaluation which will be biologically sound and at the same time not unnecessarily overregulate international embryo movement. This proposal recommends a slow approach to acceptance of 9CFR Part 93 to make certain safe procedures are in place. This is being done very responsibly by VS-APHIS and this process should be strongly supported: a) to allow consultation with OIE, the International Embryo Transfer Association, and VS-APHIS of USDA; b) allow time for public comment; c) allow time for qualifying transfer units to become operative. The American Embryo Transfer Association feels that standards should be established for cattle only and it has considerable support in the industry."(8) This is related to both the economic significance of bovines and the amount of research that has been directed to embryo transfer of this species.

While they may not cause measurable restraints to international commerce in embryos, there are as yet unresolved legal considerations.

For example, many states have enacted legislation which provides that the implied warranty provision of the Uniform Commercial Code does not apply in the sale of "livestock". It is not clear whether embryos are to be considered livestock.

Legal proceedings may result from low viability of embryos when thought to be related to improper freezing and preservation in transportation.

Questions relative to liability could be raised when a recipient animal dies containing a valuable embryo; or if she contracts a transmissible disease.

Many other questions of a legal nature will present themselves concurrent with the evolution of technology and commercial pressures.

2. Effect of New Technologies on International Commerce

In reviewing mechanisms for transferring animal germplasm, at least three systems need to be considered: 1) movement of the entire animal as a source of germplasm; 2) movement of semen from the male; 3) transfer of ova or embryos to females for the purpose of gestation. Research on disease factors related to international commerce in live animals and the usual trade commodities such as meat, has been carried out for years. The more valid, confirmed and internationally accepted research results have come from the more sophisticated laboratories in the affluent countries. This is related largely to their ability to establish the required biocontainment facilities for working on hazardous pathogens and to support qualified staff in the wake of a mandate from their livestock industries to maintain a relatively disease-free animal population, with special emphasis on freedom from FMD. The cost-effectiveness of this policy has been repeatedly demonstrated.

Some valuable research has been carried out based on a) bilateral agreements with other countries of advanced technical sophistication; b) bilateral collaboration with developing countries in which a major epizootic disease is widely disseminated in the animal population and c) through participation in programs with international agencies such as the Food and Agriculture Organization of the United Nations (FAO).

Because of the major significance of the health status of donor animal populations, it is expected that emphasis will continue to be placed, for the foreseeable future, on research with post-partum animals regarding the most important epizootic diseases. This is of consequence not only to the livestock industry of the developed countries but is also crucial to furthering agricultural self-sufficiency of the bulk of the developing countries.

Techniques developed for handling of animal semen improved over the years and have proven to be effective from the standpoint of collection, storage and transportation. Techniques to determine the disease-free status of animals prior to their entry into the artificial insemination (AI) centers are standard procedures. Bloodserum from candidate animals is subjected to immunological tests, with a view to detecting antibodies in the serum. There are however a few exceptions where antibody development is not adequate to provide detection. Alternate identification methods of the suspected pathogen are then attempted.

Much additional work is required for determining the presence or absence of disease-producing pathogens in semen, particularly in species other than cattle. It is expected that a considerable amount of this work would be carried out if the economic importance of the respective species to countries with adequate technological competence were established. At the present time, the practice is to assume that if infectious disease agents are present in semen, the disease can be transmitted genitally.

In the past, bovine semen has been imported into the U.S. from some countries with FMD, such as from approved AI centers in Germany and Switzerland. A ten percent aliquot of each semen collection was then tested at the USDA Plum Island Animal Disease Center (PIADC) for freedom from potential pathogens.

VS-APHIS of USDA is currently in discussion with a Latin American country to develop a protocol concerning the sanitary requirements to be observed in the exportation of bovine semen to the U.S. The general conditions provide that the AI center must be recognized and approved by the Animal Health Secretariat of that Government's Ministry of Agriculture and be physically isolated from any other livestock operations such as a feedlot, auction market or ranch. The center must provide facilities to isolate donor animals and operate under health and sanitary requirements established jointly by the U.S. and the cooperating Government. The agreement describes special conditions for housing of animals, the off-duty contacts that might be prohibited for personnel working in the facility, and provision for separate equipment for feed, veterinary treatment and semen collection.

The diseases receiving special attention through testing programs both before, during and after semen collection include FMD, tuberculosis, brucellosis, vesicular stomatitis, and bluetongue. In addition, certifications from foreign officials are required for lack of evidence of trichomoniasis and vibriosis in AI herd animals for 12 months and no clinical evidence of leukosis or Johne's disease for 5 years at the same facility. The proposal provides that samples collected for disease identification may be divided for testing at Veterinary Services' Foreign Animal Disase Diagnostic Laboratory (FADDL) and the other by the laboratory chosen by the collaborating country's Ministry of Agriculture. The opportunity to have semen eligible for import to the U.S. under this proposal depends primarily on the health history of the individual donor animal and the potential exposure during his lifetime. In addition, semen samples are tested for the possible presence of FMD virus and may also be tested for bluetongue virus if the country is one considered by USDA as an area where bluetongue exists.

There is little in the way of current research being carried out that implies a major modification toward relaxation of the concepts described earlier; they require reasonable assurance that the donor animal is free from demonstrable disease agents.

The more recent development of technologies relating to embryo transplant, and particularly those of the bovine species, offer intriguing possibilities of enhancing and broadening the application of improved genetic resources. The rapid development of embryo transplant centers has demonstrated the apparent economic advantages related to embryo transfer. The establishment of regional and international organizations primarily committed to fostering the application of available technology indicate future growth in the field. Established organiztions such as FAO and OIE recognize the significance of the trend and incorporate in their programs consideration of ways to increase the application of technology in international commerce, with particular emphasis on the aspects of animal health and ensuing well established advantages to improved productivity.(7)

In addition to the improved genetic capacity of the animals resulting from outstanding genetic resources available, the international movement of embryos, as opposed to live animals, is particularly attractive from the standpoint of initial cost incurred.(8) There are further major advantages in that the resulting offspring of the embryo transfer receives the maternal antibodies of the host animal residing in the country of destination.(9) It also allows the development of natural antibodies in the newborn animal as it is exposed to local environmental factors and influences.

Currently there are proposed regulations on the import of embryos into the U.S. which provide that an embryo may not be imported if there would be a reason for denying an import permit to the donor sire or donor dam under 9CFR92. This is based on research results concluding that embryos are capable of transmitting animal diseases from both the donor sire and donor dam to other animals. There are no procedures available for testing embryos to determine their own disease status. However, this premise is being challenged by the direction of current research.

Current policy further implies that if a disease should be transmitted from the donor sire or the donor dam to an embryo, such disease is considered present in the donor sire at the time of collection of the semen or in the donor dam at the time of collection of the embryo. An embryo could also carry infectious animal diseases if it became contaminated with disease organisms during or after collection. Two approaches can be taken to control disease transmission through embryo transfer: one is based on the health status of the donor animals, male and female; and the other on the health status of the embryo. This latter premise is currently receiving great attention by the numerous embryo transfer associations that have been established. The economic advantages of being able to treat an embryo as an entity from the standpoint of health certification for commerce as compared to the costs involved for carrying out required quarantine, testing and shipping of developed animals is obvious. It has been accepted that if the donor animals are free from infectious diseases, the embryos will likewise be free provided that sterile materials and aseptic procedures are used in their collection, handling and transfer.

Causal agents of infection have been isolated on a number of occasions from the fluids used to flush embryos from infected donors. It is necessary to use strict aseptic procedures for making embryo collections and meticulous care in preparing sterile instruments, fluids and containers used for flushing and collecting embryos. In addition, serum components or any additional materials used in collecting, washing, culture, freeze-thawing, micromanipulaton and transport of embryos must be mycoplasm and virus-free.(10)

As a consequence, in addtion to establishing that both the sire and the dam are free from demonstrable diseases, the actual technology of embryo collection, storage and transportation requires meticulous attention to detail from the standpoint of transmission of additional pathogens that might have been acquired during the process.

The more appealing concept is based on establishing the health status of the embryo as a living entity unto itself, and apparently free of influences of either its sire or dam from the standpoint of disease. Disease control policies based on the health status of the embryo depend on laboratory research required and supported by field trials to determine the likelihood of disease transmission by embryos under real situations. Diagnostic procedures might likewise be developed which can be carried out on embryos as individual entities. Most of the studies to date have been directed to testing the potential of embryos transmitting various pathogens. Several of these can be transmitted through embryo transfer if they are either within or on the embryo when it is transferred or in fluids transferred with the embryo. These pathogens may be transmitted through the embryo if they are in the ovum at the time of fertilization or are carried into the ovum at fertilization either within or attached to the spermatozoon or following fertilization if the pathogen passes through and becomes imbedded in, or adheres to the surface of the zona pallucida. Subsequent to this stage, the pathogen may reach the embryo as a result of damage to the zona pallucida due to manipulation or from other trauma such as imperfect freezing and thawing operations.

If embryos are properly handled during collection and subsequent manipulation, and if recommended procedures are followed for dilution, collection and washing, and if effective antibiotics are contained in washing solutions, there is a relatively short list of potentially dangerous, disease-transmitting pathogens to be considered. It seem thus indicated to direct research toward the behavior of specific pathogens known to be major deterrents to livestock production.

Results of soundly designed experiments repeated and confirmed by recognized international centers could profoundly modify current constraints to international commerce in embryos related to disease control.

Policies on international commerce in live animals have been generally established and accepted. Research carried out has been considerable and will continue, and major breakthroughs toward relaxing current health-related constraints are anticipated. The policies relative to international shipment of animal semen still are largely based on the health status of the donor animals. The technology of embryo transfer has developed to the point that there are clearly areas where research, if properly carried out, may facilitate international transfer of animal germplasm. In order to encourage legitimate international commerce in embryos, major research efforts need to be supported in order to have the best genes available without jeopardizing the health of the animal populations.

The level of technology existing in developing countries regarding the safety of importation of animal germplasm into the U.S. can be identified succinctly. For the foreseeable future, transfer of germplasm of animal origin into the U.S. must rely on procedures established by VS-APHIS of USDA as carried out in institutes authorized by that Agency, presumably in the U.S. Samples may be divided so that an officially authorized laboratory in the host country may carry out parallel tests using mutually agreed upon protocols and reagents. Current policies of VS-APHIS provide for development of such bilateral agreements for importation of semen. Hearings currently being held on proposed legislation (9CFR93) are intended to provide standards for importation of embryos based on limited research available at this time.

However it is highly essential that the U.S. assume a leading role in research that will facilitate international commerce in animal germplasm. This is an expanding industry and currently represents export earnings in excess of $25 million for this country, primarily through private enterprise.(3) By encouraging and supporting research on international commerce in animal germplasm including both semen and embryos, the U.S. will stay in the forefront of international technical discussions on research and participate in the development of guidelines for international commerce. By active participation in the regulatory programs of the international agencies such as OIE and the research and developmental programs of FAO and the International Livestock Center for Africa (ILCA), the U.S. will assist in the establishment of rational policies and divert attempts at unreasonable restraints that may be demanded by potential importing countries.

Technological advances that might alter current legal importation safeguards are those that demonstrate that embryos themselves can be dealt with as biological entities. At present, the health status of embryos is considered to be dependent on that of its parent animals. This means that criteria for allowing an embryo to enter the U.S. are based on the health status of the individual parent animals, of their herd of origin, and on the confidence ascribed to the animal health services of the country of origin. Research has been carried out on the transmissibility of some disease agents via embryos and on techniques for eliminating disease agent contamination of embryos through collection and washing procedures. With information currently available, it is expected that standards can be established for specific diseases that would enable authorization for embryo importation. If and when such standards can be established for other disease agents, similar provisions may be considered.

3. Impacts of Treaties on International Commerce.

In general, international treaties or commissions between governments, including the U.S., deal with the movement of live animals or specific animal products such as meat or semen. Restrictive requirements for commerce are generally under the jurisdiction of the respective national veterinary services because of hazards related to disease prevention and control.

The U.S. and Mexico for example entered into a convention to "safeguard the livestock interests of their respective countries through the prevention of the introduction of infectious and contagious diseases". This convention was signed by government representatives of both countries on March 28,1928, ratified by the Senate the same month, and signed by the President in April of the same year. The ratifications were exchanged in Washington in 1930. This was the major document authorizing U.S. participation in the control of FMD in Mexico between 1947 and 1953. The subsequent cooperative action between Mexican and U.S. veterinary authorities is one of the finer examples of international cooperation among scientists in undertaking a program to control a burden that was socially and economically destructive not only to the affected country but to the region and the continent as a whole.

On November 3, 1971, the Secretary of Agriculture was authorized by Congress, under PL 92-152, to develop cooperative agreements with Colombia, Panama, the countries of Central America, Mexico, the Caribbean area and Canada to work on common problems related to communicable diseases of livestock and poultry. The respective Ministries of Agriculture and animal health officials of the governments promptly endorsed the proposal, which was partially encouraged as a result of the imminent construction of the Panamerican Highway. The Highway would have created a link between South America, a continent infected with FMD, with the countries of North America which had been able to acquire and maintain freedom from the disease. Concurrently, because of concerns expressed by the U.S. livestock industries, the National Security Council advised that no more U.S. funds would be expended for the Panamerican Highway development until Colombia had organized a program that would assure control of FMD and until an FMD-free zone was established in portions of Colombia along the route planned for the Highway. This collaborative program has also been highly successful.

There are many existing bilateral agreements, at the level of the Secretary of Agriculture or the Administrator of APHIS, between the U.S. and other governments regarding import health requirements. These agreements establish requirements for animals and animal products entering the potential country of destination and provide for more precise interpretations of 9CFR for animals or animal products being considered for entry into the U.S., and depend on the effectiveness of national animal health surveillance in the country of origin.

Title 9 of the Code of Federal Regulations Part 92 contains special provisions for countries of the Western Hemisphere free of FMD regarding the importation of animals and animal products, including semen of ruminants and swine, into the U.S.

In addition, specific protocols have been prepared for importation of animal semen and embryos from countries where FMD does exist, although such protocols for embryos have never been implemented, since there is no legal authority to do so. These semen protocols have been handled on a case by case basis through mutual agreement by animal health authorities of the respective governments.

Apart from the U.S., a great number of countries has concluded bilateral agreements and conventions. Most of them have been published in the United Nations Treaties series in the original language, as well as in the English and French translations, and they are filed with FAO. The analysis of veterinary bilateral agreements is of considerable interest in the context of studies of health requirements applicable to international commerce, if they are aimed at identifying ways and means of eliminating, through international cooperation, possible trade impediments which such requirements may constitute. First, the mere fact that bilateral agreements do exist indicates that the subject of health requirements is negotiable. This is an acknowledgment that not all agreements can possibly contain provisions on every subject of concern to the potential importer or exporter. A major component in nearly all bilateral agreements are provisions related to specific, scheduled animal diseases. The relevant requirements contained in different agreements have been collated and tabulated by the Animal Production and Health Division of FAO.

The provisions of the General Agreement on Tariffs and Trade (GATT) differ from the bilateral agreements in that the provisions have been formally agreed upon on a worldwide level.

The impact of health-related measures on international commerce cannot be fully assessed because the health measures are only one part of a vast range of different factors which affect national as well as international economies. There is not only a complex of tariff, non-tariff and sanitary measures considered by importing countries but there are also certain measures taken or ignored by exporting countries such as export taxes, licensing and general policies. The role of import health requirements is obviously not quantifiable and is extremely difficult to assess. However, it must be considered that a great step forward was achieved when the principle of GATT was formally agreed upon by a large number of countries, indicating that health measures shall not be "applied in a manner which would constitute a means of arbitrary or unjustifiable discrimination between countries where the same conditions prevail, or disguised restriction on international trade" (GATT Article XX). (11)

Bilateral or regional trade agreements are normally meant to facilitate and encourage international commerce by the participating parties in order to identify, and restrict where possible, the potential for restraints. The same concept applies to bilateral agreements with certain countries concerning research on technical problems of mutual interest. Considerable expansion of such endeavors would be to the advantage of all concerned.

4. The Impact of Tariff and non-Tariff Barriers to International Exchange

Considering the relatively limited scope of international movement of germplasm, there does not appear to be any significant deterrent to international trade from the implications of tariff-related constraints. Since there is no ad valorem assessment of duties on specific products such as semen and/or embryos currently applied, this is not a barrier to commerce.

Non-tariff measures are difficult to assess considering the many ways in which they interact. In some cases, they represent a hidden menace to trade as the way in which they are used is often not predictable but they may be applied only for products originating in certain countries.

The establishment of import quotas for germplasm would not appear to be of great concern, since a major objective of its importation is an increase in agricultural production. It does not seem that there would be seasonal limitations to the collection or availability of animal germplasm. Regulative actions taken concerning international commerce in animal germplasm are primarily related to maintaining the health of the national herd and consequently fall under the jurisdiction of the national veterinary service of the respective Ministry or Secretariat of Agriculture (occasionally, Ministry of

Health). The basic concept of restrictive animal health actions relates to the fact that the defendant, in this case the animal, must prove his innocence, or freedom from disease.

The relative importance of sanitary risks and the action taken to avoid these risks depends on livestock production and health conditions in the importing country. Health requirements tend to become more stringent with the growing value and improved health of the livestock population.

Animal health requirements are bound to act as barriers against trade. They are not considered to be tariff barriers but are usually not included under the term "non-tariff trade barriers" for reasons of semantics as well as substance. As a matter of semantics, the term non-tariff trade barrier has been reserved by general usage to such purely economic and administrative measures as import quotas, seasonal limitations, licensing, tariff classification and customs valuation. Health requirements differ from these non-tariff measures by several characteristics including the objectives themselves. An important differential feature is the fact that health requirements including restrictive and prohibitive measures apply not only to international trade but are also applicable to intra-national traffic.

Another essential qualification is the fact that non-tariff measures are economically motivated and of a restrictive nature meant to protect national economic interests. Health requirements on the other hand are the concern of each individual importing country and are designed to prevent regional and worldwide spread of contagious disease. The objective of health requirements, even if constituting trade impediments, is to prevent trade which is undesirable from an international point of view.

Lack of information on the animal health status of a country is an obstacle to foreign market access. It should be emphasized that there is not necessarily a definite correlation between restrictiveness and effectiveness of animal health requirements. It is not the restrictiveness but rather the appropriate selectiveness of restrictive requirements which determines the efficacy of health requirements. By emphasizing this characteristic it is feasible to reduce the unintentional barriers resulting from health requirements, and, at the same time, maintaining or even intensifying the intentional and well designed barriers against disease.

It is apparent that the impact of health measures on trade cannot be accurately assessed because of the interaction of this factor with a multiplicity of primarily non-tariff barriers which affect national and international livestock economies.

The cost/effectiveness of technically sound and politically supported import health policies has been described in chapter 1.

With improvement in diagnostic procedures that will permit authorized movement of animal germplasm, it is anticipated that other barriers may present themselves. This seems particularly probable in countries of the European Common Market which have developed a surplus of dairy commodities. Even though this may have been the result of importation of superior animal germplasm from the U.S. over the past several years, their commitment to subsidized agriculture within the European Community implies further efforts to augment their potential for international commerce in animals and animal germplasm. This is often accomplished at the expense of equivalent or superior U.S. resources. If the U.S. can establish and maintain an apparent and hopefully real technological superiority in the development of disease identification techniques, much will be accomplished toward alleviating the otherwise negative restraints against the U.S. that might develop from other countries or groups of countries in the international exchange of germplasm.

It is generally accepted that international movements of germplasm poses considerable advantages to improved animal productivity and that the barriers which may appear can be negotiated satisfactorily.

Considering the value of the livestock populations at risk, it is not expected that there would be an intentional shift in the balance between promotion and protection.

5. Opportunities for Augmenting Animal Germplasm Exchange

At present, and in the foreseeable future, major considerations to determine the potentials for commerce in animals and animal products are specified by the importing country and are related to three overriding factors: 1) the value of the population at risk; 2) the level of their susceptibility to disease; 3) correlation between productive efficiency and freedom from major epizootic diseases. In response to the collective judgment of the respective livestock industries, the national veterinary service establishes the protective mechanisms considered commensurate with those factors, in relation to the economic requirements of government policy.

There are circumstances when governments will risk importation of animals and animal products including meat from countries where major epizootic diseases such as FMD occur, in view of cost advantages to the consuming public. Even though this action may increase the hazards of importing the disease to the livestock population, the overall cost of eliminating occasional outbreaks may be considered viable as a political decision. In general, the cost effectiveness of preventing disease introduction to the extent possible, will require major constraints by the application of health requirements for importation of animals and animal products.(4)

At the May 1985 public hearing in Kansas City, submissions were made by representatives of the major industries affected by the regulation proposed by Veterinary Services, APHIS-USDA. The major objectives of some of the agricultural interests are the establishment of standards to allow embryos to be considered as entities in the promulgation of regulations for international commerce as opposed to requirements as currently stated, based on the health status of the embryo's parent animals. The group representing zoological animals was particularly concerned with the requirement that embryos considered for importation would be limited to those derived by artificial insemination, their contention being that, with some species, this is not an available technology.

It is anticipated that Veterinary Services will hold another hearing with proposals reflecting the concerns expressed at Kansas City or will modify the existing proposal to accommodate the concerns expressed.

Tentative international policies relative to embryo transfers submitted to the OIE meeting in Paris in May 1985 have not yet been acted upon. However, a Committee was established to work on the development of standards applicable to international commerce in animal embryos. The committee is scheduled to meet in Paris in December 1985 for the purpose of assembling a proposal that will be submitted to the convening body of OIE in May 1986. The U.S. Delegate to the OIE is the Chairman of this Committee.

It is imperative that the U.S. take action on establishing standards permitting importation of animal embryos in view of the pressures to promote such commerce; it will take place either with or without official sanction unless reasonable provisions are made for its legal implementation.

It is accepted that any modification in U.S. policies relative to international movement of germplasm will depend on authorizing legislation resulting from either discussion at public hearings called by the regulating agencies or be mandated by Congress. Such action would only be predicated by new technical breakthroughs.

Research dealing with international movement and sources of germplasm such as whole animals or semen has continued at a fairly consistent level for a long time. It appears that similar levels of reseach support will be maintained. There are however areas where research concerning disease transmission by international exchange of animals and semen could provide valuable information. At the present time, the major criteria for establishing potential presence or absence of disease in an animal relate to the serological identification of antibodies produced as a result of a presumed offending pathogen. It is well established that a high percentage of animals with demonstrable antibodies in blood may not and will not necessarily have clinical disease attributable to that pathogen (12). It has often been said that it is safer to have animals with circulating antibodies indicating a level of protection than animals which are totally susceptible. This "after the fact" search for antibodies as evidence of exposure to a pathogen is a major deterrent to the export of animals and semen from North America. Even though the North American Continent enjoys freedom from the major epizootic diseases, the presence of antibodies to the lesser pathogens that may not exist in livestock populations in other countries, is given as grounds for prohibition for importation into those countries where such pathogens may not have been identified.(13)

The development of technology to identify presence or absence of pathogenic agents, rather than an indication of the presence of circulating antibodies that may be the result of exposure to a virulent pathogen or to an effective vaccine, would provide vast relief to the export potential of U.S. animals and animal products including semen and embryos.

Much research is needed to determine more accurately the relationship between infectious disease-causing microorganisms and the transmission of the diseases which they cause by infected semen. Support for such research would require determing cost/benefit ratios for the level of research and the number of species to be considered. At present, it appears that it is safer and cheaper to assume that if infectious disease agents are present in semen, the disease can be transmitted genitally (10).

With the newness and spectacular expansion of embryo transfer (ET) and its application to commercially viable processes, it is understandable that major emphasis needs to be directed to keeping pace with this burgeoning industry.

One of the major requirements which is not in the nature of research but rather concerns the development of discipline within the embryo transfer industry, is the establishment of an international body to identify a) standards relative to quality control from the standpoint of germplasm viability and b) standards relating to health requirements in international commerce. This could relate to establishing criteria for ET Centers qualifying for official recognition.(14) Standards need to acknowledge that pathogens can be transmitted through ET if they are either within or on the embryo when it is transferred, or in fluids transferred with the embryo. In order to be effective in participating in the development of international standards, the U.S. itself should establish an oversight board responsible for setting guidelines for minimum standards. Such a board would incorporate representatives of the ET industry, research scientists and the respective regulatory agencies; it should also include representatives from Canada and Mexico.

More specific and detailed biological research concerning embryo transfer is described in the paper by W.C.D. Hare presented at the May 1985 OIE meeting in Paris. (10). It is expected that there is sufficient impetus to guarantee an at least moderate research investment in U.S. institutions with broadly qualified staff to assure participation of not only physiologists but animal health scientists. Their primary function would be to deal with diseases that are indigenous to the U.S.

It will also be necessary to undertake much more research on the role of embryos and their potential to transmit exotic pathogens. By law, regulations, or policy this work can only be carried out on such pathogens in institutions with appropriate biological security. It is expected that optimum benefit would result if studies at this level were run in collaboration with institutions in other countries with similar technical and biological competence, and particularly in countries where there is a national mandate for involvement in the international traffic of animals and germplasm. This action would help considerably in assuring broad agreement on standards acceptable for international commerce.

Another mechanism that must be incorporated because of its potential for cost effective research on exotic pathogens, is the development of cooperative agreements with developing countries where these diseases are prevalent in the livestock populations.

The Office of International Cooperation and Development (OICD) of the USDA is at present holding discussions of mutual interest with Pakistan and Yugoslavia on research in embryo and germplasm transfer.(15) USDA, APHIS, VS also is actively cooperating with onsite veterinary guidance. OICD activities could be expanded considerably to include work with other developing countries, including Latin America, where statistically valid protocols could be drawn up which might provide answers to the possibility of treating embryos as individual entities.

Recognizing that the major barrier to international commerce in animal germplasm is related to maintaining the national herd free of exotic pathogens, there is little likelihood that there will be legislative modifications in this policy until research is carried out and validated under varying management and epizootiological conditions. It is imperative for the U.S. to develop and maintain an increased level of research toward that end, not only to enhance the potential for importing genetically desirable species but also to identify greater potentials for export markets.

U.S industry has demonstrated its ability to provide some technology transfer in embryo collection and implantation techniques to developing countries.(16) This should be encouraged and supported where appropriate. There is opportunity and need for much more functional participation by U.S. scientists and administrators in the work of existing international organizations such as FAO, committed to the improvement of animal production and health. It is similarly anticipated that recently acquired U.S. membership in OIE will assist considerably in the establihsment of technically valid and politically acceptable standards for international commerce in animal germplasm. Active participation of U.S. scientists in international and regional work concerning the improvement of animal production and health, provides greater assurance that standards developed will be scientifically valid, rather than being adopted arbitrarily. (17)

Current legal/regulatory options available to Congress include defining the basic responsibilities of Veterinary Services, APHIS-USDA and the passing of specific Acts relative to maintaining the health status of the U.S. livestock industry. Veterinary Services of APHIS-USDA have held hearings on proposed regulations concerning the importation of embryos. Based on the comments presented at these hearings, modifications in the proposal are under consideration. As a consequence, the proposal may be modified to the extent that another hearing may be necessary.

It will be highly desirable if provisions were made for the establishment of a Board or Panel composed of a) scientists interested in the development of the industry of embryo and germplasm transfer, b) those responsible for the biological aspects of relevant research, c) representatives of the animal health research group associated with embryo transfer and d) scientists responsible for developing minimum standards for quality control and viability of embryos that are manipulated according to demands of the industry. (Page 36)

It is likewise desirable for Congress to mandate active U.S. participation in international research directed toward the development of information that will permit safe and more effective international commerce in germplasm. Some research would best be carried out in developed countries and some in countries where major diseases exist.

In addition to its function of providing funds to the United Nations and its specialized agencies, Congress should take steps to encourage active participation of U.S. scientists and administrators in FAO in order to assure more effective application of FAO's declared objectives of developing better conditions for exchange of animal germplasm.

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December 17, 1986

Ms. Carolyn Swann
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Dear Ms. Swann:

We sent a manuscript to you on November 25, 1986, entitled "Technologies to Maintain Animal Germplasm in Domestic and Wild Species" by B.L. Dresser and S.P. Leibo. We have since found errors on several of the pages and would like, if possible, to include these corrected pages in the manuscript.

Any help you can provide will be very much appreciated.

Sincerely,

Vickie Stidham

Vickie Stidham
Secretary to Dr. Betsy L. Dresser

cc: S.P. Leibo

Encl

vs

NOV 26 1986

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November 25, 1986

Carolyn Swann
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Dear Ms. Swann:

Susan Shen requested that I send the attached manuscript entitled, "Technologies to Maintain Animal Germplasm in Domestic and Wild Species" to your attention. If you have any questions, please call my office.

Thank you.

Most Sincerely,

Betsy Dresser

Dr. Betsy L. Dresser
Director of Research
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NOV 26 1986

TECHNOLOGIES TO MAINTAIN ANIMAL GERMPLASM IN DOMESTIC AND WILD SPECIES

By

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Cincinnati Wildlife Research Federation

and

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Prepared for the Congress of the United States, Office of Technology Assessment for Evaluation of Technologies to Maintain Biological Diversity.

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INTRODUCTION

Nature, by means of biological evolution, has devised mechanisms by which a rather stable gene pool of a species is maintained, while providing means by which alterations of this gene pool can be introduced and incorporated. Early in his own evolution, man sought to alter these relationships in other species by "domesticating" a variety of animals. Throughout history, human societies have become increasingly dependent upon domesticated animals both as aids in performing work, e.g., horses, cattle, camels, water buffalo, and elephants, and as food itself for human consumption, e.g., hogs, cattle, sheep, and goats. Humans use many species of domesticated animals both as "helpers" (dogs to hunt and to shephard sheep and goats, for example) and for their own contentment and amusement (dogs as pets and as performers). Man has even succeeded in turning certain species of "pests" such as mice and rats to his own ends by domesticating them for research purposes.

As a consequence of man's ability to exploit other species, both positively and negatively, however, he has introduced yet other problems. Not the least among these is the total loss of a species that becomes extinct as a result of human "control" of its habitat, its food supply, or its reproduction. Even without causing the total extinction of a species, however, man's control of domestic animal reproduction portends untoward consequences. That is, all biological systems change with time. Reproduction of naturally breeding populations of animals apparently permits changes to occur gradually, certain ones to become permanently fixed in the population at large, others to be lost by microevolution. In this way, animal species adapt to environmental changes. Human intervention, however, clearly alters this natural order. By animal husbandry to control reproduction, humans can "breed out" or "breed in" certain characteristics judged to be desirable. Some of these characteristics may be apparently superficial and of no great consequence, e.g., coat color or horn length. Others may be of enormous apparent value, e.g., butter fat content of milk or disease resistance. It should be noted that the qualifier "apparent" has been used to describe such characteristics. The reason is that, whereas the phenotype of an animal may be relatively easy to measure, e.g., pounds of milk produced per day by a lactating dairy cow, the genotype is much more difficult to "measure" at the present time with any precision. Therefore, the elimination of a specific phenotypic trait by selective breeding may, at the same time, cause the loss of several other unrecognized traits carried on genes closely linked to that "undesirable" one.

In short, the husbandry of domestic animals, like so many other human activities, represents an empirically derived compromise between benefits and risks. The benefits are numerous and, for the most part, obvious. The risks are more subtle and often unrecognized. One almost certain risk will be the loss of biological diversity by domestic species. As their reproduction is controlled, the gene pool of domestic animals will shrink. In a sense, the majority of animals in a controlled population will become more alike. As a consequence, there will be a loss of heterozygosity with a gradual reduction in the diversity of the population as a whole. Several examples illustrate the danger. Researchers who study the genetics of inbred strains of laboratory mice have long recognized that the breeding of mice to maintain a specific mutation often results in the gradual loss of fecundity of the strain. That is, hybrid strains of mice normally produce litters of 8 to 10 mouse pups. Mutant strains that have been inbred over many generations may produce as few as 2 or 3 pups. This loss of fertility may become so severe that it is often difficult to maintain the strain at all. Another often cited example is found in zoo populations of wild animals. Given a very limited number of captive males, zoos often exchange either semen from a few males for artificial insemination or the males themselves, which are used to breed captive females. The offspring of these controlled matings are then used for subsequent matings. Often, however, these matings are between closely related individuals. As a result, there has been a demonstrable decline in the overall vitalilty of zoo populations of certain species.

The situation for animal populations in the wild is, if anything, even more difficult. The greatest threat to the preservation of genetic diversity of wild animals comes from the destruction and degradation of their habitats (IUCN, 1984). It has been estimated that if today's pace of habitat destruction continues, one million species of plants and animals may become extinct by the end of this century (AAZPA, 1983). Continued destruction of tropical rain forests, the most species-rich land environment on earth, may result in mass extinctions that would permanently impoverish the planet.

Other threats to species come from the destruction of animals and plants for food trade. The Global 2000 Report to the President of the United States (Barney, 1982) states that the extinction of biological species projected for the coming decades will be largely human-generated, and on a scale that renders the gradual process of natural extinction trivial by comparison. Extinction is a catastrophe, not only aesthetically or because of the effect it has on the ecological balance, but also because it deprives Mankind of part of Nature's potential. Each species forms a genetic reservoir (genome) which may be of value in agriculture, medicine or industry (Daniel, 1981). Forty percent of our present-day medicines are derived from wild plants and animals (Wolkomir, 1983). It is impossible to know what other genetic resources lay waiting to be tapped.

Animal germplasm should be preserved not merely for altruistic reasons, but also for reasons that are of direct benefit to mankind. It is not known which plants and animals may prove useful in the future. It is known, however, that certain animals have proven invaluable to our knowledge of diseases and human health. For example, armadillos have served as a tool to study leprosy, as they are the only animal model to acquire this disease when injected with Myobacterium leprae experimentally (Storrs et al., 1979). The small cotton top tamarins (Saguinus oedipus) from South America have the highest incidence of colonic cancer of any animal, and have proven to be an excellent species for the study of this fatal disease (Lushbaugh et al., 1984). Spontaneous diabetes mellitus in the South African hamster (Stuhlman, 1979) and A.I.D.S. (acquired immune deficiency syndrome), the viral disease now reaching epidemic proportions in man may have potential cures because of wild animal species that have been chosen for study (Letvin and King, 1984). Hepatitis B virus was discovered in a group of North American woodchucks (Marmota monax) by a zoo pathologist at the Penrose Research Laboratories in Philadelphia (Snyder et al., 1982). The study of the livers of these animals has given insight into the etiology of this often seen liver tumor. There are many more examples.

But, just as the control of animal reproduction by humans has caused the problem, research may provide some relief. The reason is that control of animal reproduction had, in the past, meant simply the selection of breeding partners based on sometimes superficial, phenotypic characteristics. Control of animal reproduction in 1986 means substantially more than that. It has become a sophisticated procedure by which not only can a desirable trait be introduced into an animal's genome, but one in which the fundamental biological mechanisms are beginning to be understood.

In addition, new interest has been generated in the field of wildlife reproduction, fueled by the groundwork laid by the agricultural industry. Zoological researchers have begun to study the reproductive processes of wild animals, and to use techniques such as embryo transfer and artificial insemination to attempt to improve the reproductive potential of wild animals (Durrant et al., 1986; Wildt et al., 1986). Zoos throughout the world have begun to assume the role of conservators, rather than merely of displayers of wildlife. According to Kurt Benirschke of the San Diego Zoo (Begley et al., 1982), zoos have recognized the urgency of the plight of the world's endangered species, and have assumed custody of them, thereby becoming the last bastions for the propagation of wild animals.

One of the biggest problems plaguing zoos, however, is the loss of genetic diversity due to inbreeding. This is particularly true with species whose numbers have been so depleted so as to cause a "genetic bottleneck." For example, the douc langur, a primate from Southeast Asia, is now virtually extinct in the wild, and there are not enough in captivity to ensure the genetic diversity necessary to keep the species alive (Gorman, 1980). Without unrelated bloodlines, they can pass only a limited array of genes to their offspring, and as a result, they gradually become genetic carbon copies which lack the built-in adaptability to environmental change which would otherwise occur through natural selection in a genetically diverse group.

The purpose of this review is to discuss reproductive biology as it applies to the maintenance and long term preservation of domestic and wild animal germplasm of mammals. Much ongoing animal research is geared toward the improvement of genetic and species diversity. The development of advanced reproductive technology such as embryo transfer and gamete preservation may represent the real key to the future for many species that are currently threatened by extinction. The major areas to be addressed are the control of reproductive processes, embryo transfer, cryopreservation of embryos and sperm, artificial insemination, *in vitro* fertilization and genetic engineering of gametes as they relate to both wild and domestic animal species.

CONTROL OF ANIMAL REPRODUCTION

A. Domestic species

The control of reproduction in many species of domestic animals has become commonplace. With few exceptions, the female of domestic species exhibit a regularly recurrent period of ovulation or estrus, in which they are receptive to breeding by the male. Since semen of certain domestic species can be successfully frozen, artificial insemination of the estrous female with frozen-thawed semen is a widely practiced procedure. Given that capability, control of animal reproduction has become increasingly sophisticated and precise. That control has now taken many forms. These can be summarized in the following way.

1. Synchronization of Estrus. Over a period of many years, substantial understanding of the physiology of the estrous cycle has been achieved (see reviews by Foote, 1978; Hansel and Convey, 1983). Particularly in cattle and sheep, this understanding has led to the development of several efficient methods for regulating the time of ovulation. For example, by administration of a combination of prostaglandin F2 (PGF) and progesterone (either by injection or in the form of implants), it is now possible to control ovulation rather precisely. This means that cows and ewes can be efficiently managed for artificial insemination programs. A variety of products are now available commercially to control ovulation in cattle. These include Synchro-Mate-B (norgestomet + estradiol; Searle Agriculture), PRID (progesterone-impregnated silastic coil or sponge, inserted into the vagina; Abbott Labs, Inc.), Lutalyse (prostaglandin F2; Upjohn Co.), and Estrumate (prostaglandin analog; Imperial Chemical Industries, Ltd.). Ovulation in sheep can be controlled by various progestational compounds. In addition, injection of PMSG (pregnant mare serum gonadotropin) together with prostaglandin F2 has also been used successfully for estrus synchronization in sheep. It is known, however, that PGF when used alone is not effective. According to Hansel and Convey (1983), "the most promising method for controlling estrus and ovulation in sows is by use of allyl trenbolone." It seems reasonable to assume that the future research on the estrous cycle will

yield methods for the control of ovulation in females of a variety of both domestic and non-domestic species.

2. Artificial Insemination with Frozen Semen. The freezing of mammalian spermatozoa for use in artificial insemination (A.I.) has become an extremely powerful tool in the control of animal reproduction. The literature in this field has become very large indeed, and numerous reviews have been published (Foote, 1980; Graham, 1978; Pickett et al., 1976; Iritani, 1980). Artificial insemination of cattle, in particular, has become an absolutely routine procedure. For example, Iritani (1980) surveyed the use of frozen semen for A.I. in 38 countries. He reported that almost 46,000,000 cows were inseminated with frozen semen during 1977-78 alone. In many of the countries surveyed, virtually all of the inseminations were performed with frozen semen. It seems reasonable to conclude that the freezing of bull semen is a reliable and established procedure. The situation with other mammalian species is far different, however. For example, Graham et al. (1978) reviewed several field trials for pigs, and reported that pregnancy rates of from 30 to 63% have been achieved with A.I. using frozen boar semen. The situation with horse semen is similar. That is, Iritani (1980) reported that several field trials have achieved conception rates of about 65% in mares inseminated with frozen stallion semen. The freezing of ram semen is somewhat more reliable. Rather extensive trials (reviewed in both Iritani and Graham et al.) have reported conception rates of ewes of close to 80% after A.I. with frozen semen. Despite these successes, and with the notable exception of cattle, A.I. of most domestic species with frozen semen cannot yet be considered a commercially successful procedure.

Nevertheless, successful freezing of semen of a wide variety of mammalian species has been reported. Among those species in which live young have been born following A.I. with frozen semen are the following: goat, buffalo, rabbit, cat, dog, fox, mouse, and wolf. Clearly, additional research is required to elucidate the causes of freezing damage, and to develop techniques to freeze semen from many species successfully.

3. Superovulation. One key element to the control of reproduction is the fact that it is now routinely possible to induce superovulation in female mammals. The practical importance of this technique is most dramatically illustrated by results in cattle. The cow normally ovulates a single, or occasionally two, ova per 21 day estrous cycle. By the injection of pregnant mare serum Gonadotropin (PMSG) or porcine follicle stimulating hormone (FSH), this number can be increased enormously. For example, a summary by Elsden and Betteridge (1977) reported that superovulated cattle routinely yielded 10 to 15 ovulations per treated donor. Some authors, e.g. Gordon (1976) have reported as many as 18 or 20 ovulations per donor. And virtually all workers in the field have occasionally collected as many as 40, 50, or even 75 ova from a single donor. This is balanced by the fact that about 20 to 30% of all treated females produce no ova at all in response to superovulation. Of more importance than the number of ovulations per treated donor is the number of embryos of transferable quality. These range from a low of about 2 or 3/cow to highs of 8.5 (Elsden and Betteridge, 1977) and 9.6 (Trounson et al., 1983) (Both citations in Elsden and Betteridge, 1977). The most important statistic of any superovulation treatment is the

number of pregnancies that result from transferred embryos. Seidel (1981) reported that three courses of superovulation of 32 apparently normal donor cows yielded a total of 328 pregnancies. That is, an average of 3.4 pregnancies per treated donor cycle were produced.

B. Wild Species

The key to progress in the field of wild animal research lies in the ability to gather enough information to understand the reproductive processes of wild animals. Technologies such as embryo transfer, artificial insemination and cryopreservation will certainly become more important as time goes by, but the use of this technology in wild species is presently hampered by a lack of basic knowledge. Extrapolation from domestic models to wild species has been helpful as a starting point, especially with wild bovids; however, it is really not reliable because reproductive processes, such as the length and frequency of estrous cycles, normal hormone levels, an animal's response to exogenous hormones, and the viability of germ cells, seem to be very species-specific (Lenard, 1981). For example, Keith Hodges, of the London Zoo, has discovered that the hormone progesterone, often a reliable indicator of pregnancy in other animals does not rise in the giant panda until the third trimester of gestation (Hodges, 1984). This means that basic information often needs to be gathered anew with each species that is studied. Added to this are the serious problems of physical access to the animals (e.g., the need for restraint or anesthesia even for the most simple procedures, such as drawing blood samples), and the lack of "scrub" animals (e.g., the value and availability of most wild animals is such that there are very few "expendable" individuals or a particular species on which to practice initial techniques as there are in the agricultural industry).

A practical, reasonably simple, and relatively inexpensive method for monitoring reproductive events is being developed, the urine hormone assay (Loskutoff et al., 1981). This test allows the researcher to establish normal hormone levels for cycling and pregnant animals which, once established, help to confirm ovulation, predict optimal times for insemination, diagnose the etiology of reproductive dysfunction and detect pregnancy. To establish normal values, daily random urine samples are collected over a period of time and tested for the presence of pregnanediol-3-glycuronide (PdG), a progesterone metabolite which has been shown to accurately reflect luteal activity. The PdG levels are then indexed by creatinine content to compensate for variations in fluid intake and output. This urine hormone analysis offers a very accurate, non-invasive method to monitor ovarian function in exotic animal species. However, it has only been tested on a few species such as the rare okapi.

An enzyme immunoassay (EIA) for estrone conjugates has also been described (Czekala et al., 1986) and applied to urine samples from a female Indian rhinoceros, a female gorilla and a female lion-tailed macaque. EIA measurements provide information that is comparable to values obtained by radioimmunoassay. Thus, this type of assay can be used to evaluate female reproductive status of zoo species without the need for a traditional endocrine laboratory. Other similar assays have been applied for ovulation detection in the marmoset monkey (Harlow et al., 1984), ovulation and implantation in lion-tailed macaques (Shideler et al., 1983), pregnancy detection in tapirs (Kasman et al., 1985), ovarian function in exotic ungulates (Loskutoff et al, 1982), reproductive studies in gorillas (Mitchell et al., 1982; Nadler and Collins, 1984; Watson, 1984), oryx (Durrant, 1983) and cheetah (Phillips et al., 1982; Wildt et al., 1981; Wildt et al., 1983 a and b; Wildt et al., 1984).

Infertility is a major problem in today's zoo animals. Oftentimes, it can be traced to extraneous factors such as light, temperature or nutrition, or to factors such as age, disease, or social or behavioral influences (Martin, 1975). Whenever possible, it has been found helpful to mimic the natural environment to evoke the optimal reproductive response. This is, of course, not always possible in a captive environment. Gorillas have traditionally been difficult to breed in captivity (Douglass and Gould, 1981). There seems to be a lack of fertile breeders, and an above normal incidence of testicular degeneration in the captive male gorilla population (Dixson et al., 1980). No cause for this problem has yet been pinpointed, but some aspect of the state of captivity is likely to be a contributing factor. Female gorillas are also prone to fertility problems, either because of a failure to breed, or a failure to conceive. Gorilla menstrual cycles have been charted somewhat accurately at various research facilities through urine hormone assays, or the use of reagent strips in urine samples to test menses (Nadler et al., 1983; Nadler and Collins, 1984; and Martin et al., 1984). Daily urine samples are presently being collected at the Cincinnati Zoo from two infertile females for hematesting and radioimmunoassay in an effort to plot their menstrual cycles. Once enough information is gathered about the nature of their cycles, a fertility drug, Clomid (see Appendix I), will be administered in an attempt to enhance their ovulation response.

A new treatment regime for female animals that are infertile because of hormonal defects has been somewhat successful. Gonadotropin hormone-releasing hormone (GnRH, see Appendix I) is a synthesized hypothalamic hormone which causes the release of the pituitary hormones that cause ovulation. It has been effective in anovulatory women, monkeys, and sheep, and may be effective with most animals. A serious shortcoming, however, is that the frequency of treatment required for this hormone is about 12 times per day. A non-stressful, automatic delivery system has been developed for administering this hormone to wild animals (Lasley and Wing, 1983). Alternating units of hormone and mineral oil are injected into polyethylene tubing attached to a small osmotic pump which is implanted subcutaneously so that it can deliver the fluids by osmosis. The pump has shown promising results with members of the cat family by promoting the birth of two cubs to a previously subfertile cheetah that was treated at the San Diego Wild Animal Park (Lasley, 1985).

If a captive breeding pair is unable to conceive offspring today, it is not so much the hopeless case that it was in years passed. Male infertility can be evaluated through semen analysis and female infertility can be evaluated through hormone studies. Problems often can be identified through these tests, and sometimes even corrected. However, a word of caution. Endocrine analyses, behavioral observations, exogenous hormone treatments, etc. have only been applied to a few species of wild animals in recent years, and very few offspring have been produced as a result of their application. To date, changes in management (environment, diet, etc.) have probably been more successful in helping to produce offspring. It is evident that much more work on improving technology needs to be undertaken (Dresser, 1984a).

EMBRYO TRANSFER

Embryo transfer is a technique by which fertilized ova and early embryos are recovered from the reproductive tract of a donor female, the genetic mother, and are transferred into the tract of a recipient female, the foster mother, in whom the embryos develop into full-term fetuses and live young. The first successful transfer of mammalian embryos was performed in the rabbit by Heape (1891). His observations stimulated relatively little further research until about 1950. Since then, there has been an explosion of research in this area. Numerous reviews and textbooks have been published that describe both the methods and the fundamental principles on which the technique of embryo transfer rests (recent reviews in Betteridge (1981), Mapletoft (1984), Sreenan (1983), Seidel (1981); texts: Adams (1982), Daniel (1978), Cole and Cupps (1977)). Although the specifics of the methods depend upon the species used, the general principles are the same whether performed in laboratory animals such as mice and rabbits, or in large domestic animals such as horses and cattle, or in wild species such as baboons and antelope.

Embryo transfer in two species, mouse and bovine, has become absolutely routine. It is not exaggeration to state that tens of thousands of living mice and cattle have been produced by embryo transfer. In general terms, the transfer of embryos of other species has been modeled on techniques devised for mice and cattle. Although the numbers of live young are somewhat less, probably thousands of live rabbits, pigs, and horses have been produced by embryo transfer. The application of successful embryo transfer to wild species is a relatively recent event, however. Its chronologic history can be highlighted as follows: Non-human primate surgical embryo transfer in a baboon (Kraemer et al., 1976); a wildlife surgical interspecies embryo transfer between mouflon (wild sheep) and domestic sheep (Bunch et al, 1977); a second surgical transfer of an embryo from a wild species into a domestic species (gaur to Holstein) (Stover et al., 1981); nonsurgical embryo transfer performed in 1983 with eland antelope (Dresser, 1985); nonsurgical embryo transfer with eland antelope involving frozen embryos (Kramer et al., 1983); primate interspecies embryo transfer (*fascicularis* to rhesus) following *in vitro* fertilization (Balmaceda et al., 1984); cryopreservation and transfer of a baboon embryo (Pope et al., 1984); frozen embryo transfers in nonlaboratory species on primates were in the common marmoset (Hearn and Summers, 1986); nonsurgical interspecies embryo transfer between two different species of wild animals (bongo to eland). These bongo antelope embryos were brought from Los Angeles to Cincinnati and transferred fresh, 12 hours post-collection (Dresser et al., 1984b). Embryos from a wild species had never before been transported long distances; nonsurgical interspecies embryo transfer from zebra to horse (Foster and Bennett, 1984; Bennett and Foster, 1985); long-term frozen embryo transfer in a wild species when an eland embryo previously frozen for 1 1/2 years was successfully transferred nonsurgically to an eland surrogate (Dresser et al., 1984c);interspecies embryo transfer from Przewalski's horse to New Forest pony (Kydd et al., 1985); embryo transfer in Dall sheep (Mehren, 1986).

Synchronization of the donor and recipient animals in embryo transfer can be accomplished through precisely timed injections of prostaglandins, such as Lutalyse and Estrumate. These hormone analogs serve to stimulate the ovaries to begin a new cycle. Superovulation of the donor is accomplished through the injection of fertility hormones such as follicle stimulating hormone (FSH). Superovulation has been fairly successful with the ungulates, but optimal drugs and dosages have yet to be refined in most other species. As many as 31 embryos have been collected from one FSH-stimulated eland cow (Dresser et al., 1983a). On the other hand, fertility drugs seem to have little or no effect on the equids (such as zebras and Przewalski's horse) (Hearn and Summers, 1986). Clearly, the hormone regime which produces the optimal superovulation response within a given species seems to be fairly individualized and much work is needed in this particular area.

In felines, the superovulation of donors and the synchronization of donors and recipients is complicated by the fact that most cats are induced or reflex ovulators, meaning that they ordinarily do not ovulate without the stimulation of copulation. Human chorionic gonadotropin (HCG) has been administered to domestic cats to cause ovulation to occur (sometimes in conjunction with stimulation by a vasectomized male) (Bowen, 1977). Researchers in several institutions have been working on embryo transfer in domestic cats with limited success, but little work has been done thus far on wild cats (Reed et al., 1981; Kraemer et al., 1979). It is hoped that domestic cats may be able to serve as surrogates for incubating embryos from small endangered wild cats such as the black-footed cat (Felis nigripes). A great deal more work needs to be done in this area to determine the best regimes for stimulating cat's ovaries to produce more than the usual number of follicles and to cause the ovulation of these follicles thus producing embryos.

The development of embryo transfer techniques is essential if genetic diversity within captive populations is to be maintained. The ability to introduce new bloodlines into a captive population through the transfer of non-local embryos into surrogates would be far preferable to the transport of adult animals for breeding purposes, or to the depletion of wild herds to add new breeding stock to captive populations. In addition, it is a goal of many zoo researchers to develop interspecies embryo transfers to the point at which embryos can be collected from endangered species, and transferred to surrogates of a more common species, thereby greatly increasing the reproductive potential of the donor species.

Other important benefits result from embryo transfer. It has been found that disease transmission between different populations can be dramatically reduced by embryo transfer (see Hare, 1985 for review). This happens because the intact embryo collected from a diseased mother is almost always free of the microbial or viral disease agent, and does not transmit the disease to the foster mother. Alternatively, the surrogate mother may confer passive immunity to her offspring that develop from transferred embryos. This may occur either through the placental blood supply or via the colostrum, the first milk. That is, it has been found that cattle of a given breed may quickly succumb to local diseases when imported into a new location. However, live calves produced by transfer of embryos from a foreign breed into a second domestic breed will usually be as disease-resistant as the domestic breed. This "accident" of biology should have important consequences for the transport of both domestic and wild species from one location to another.

Interspecies embryo transfer has enjoyed limited success in wild animals, but much more research needs to be done in this area (Kraemer, 1983). The list of failures (eland to cow (Dresser et al., 1982b), water buffalo to cow (Drost, 1983), tiger to lion (Reed et al., 1981)) far exceeds the list of successes. It seems that most embryos can develop to the early blastocyst stage in the oviducts of unrelated specie (Daniel, 1981), but further development requires a much closer relationship between donor and recipient and similarity in time and type of implantation, placenta formation, rate of ovum transport, length of gestation, birth weight and neonate and maternal post-partum behavior. Several techniques have been tried to help overcome the surrogate mother's immune response and prevent the rejection of the "foreign" embryo in an interspecies embryo transfer. Recently, a domestic horse mare gave birth to a donkey foal after she had been injected with donkey white blood cells (Antczak, 1985). Prior to this treatment, all other donkey embryo transfers into domestic horses had failed. It is not yet clear why the procedure was successful and the methods and results of this experiment have not yet been published in a scientific journal. A second ploy might be to transfer a native embryo into the surrogate along with the foreign embryo, the thought being that it will help to set up the environment necessary to maintain pregnancy (Lenard, 1981). This technique is, of course, only feasible with species that routinely experience multiple births and has, thusfar, remained a thought !

Although various attempts have been made at embryo collection of non-human primates at various zoos and primate centers over the years, very few of these procedural attempts have been reported in the scientific literature. For a review of the existing literature on superovulation and ova collection attempts in the more common non-human primates, see reviews by Brackett (1981) and Kraemer et al. (1979) and recent papers by Hodgen (1983), Clayton and Kuehl (1984) and Bavister et al. (1985).

In summary, then, embryo transfer has become a widely used technique to produce living young animals, especially of domestic species. Although it is as yet still a novel procedure in wild species, continued research will inevitably make transfer of wild animal embryos as successful as domestic animal embryos.

IN VITRO MANIPULATION OF MAMMALIAN EMBRYOS

In addition to dozens of reviews and monographs devoted to many aspects of those disciplines, several journals are now published whose principal focus is embryo manipulation and transfer. These include *Journal of Reproduction and Fertility*, *Biology of Reproduction*, *Gamete Research*, *Theriogenology*, and *Journal of In Vitro Fertilization and Embryo Transfer* (abbreviated IVF). As in any discipline, articles also appear regularly in other less specialized journals as well. The International Embryo Transfer Society also issues a regular Newsletter in which a bibliography of the literature describing this discipline is published. In short, the literature describing early mammalian embryology and reproduction is growing at an astonishing rate. It would be impossible to summarize this wealth of scientific understanding concisely. Therefore, the balance of this review will address relatively few aspects of the entire discipline, and will be confined to those areas that are changing most rapidly or that have reached a level of reliability so as to be considered relatively routine procedures. All of the procedures and techniques to be discussed below depend upon the capability to produce live young from embryos that have been manipulated *in vitro*. That is, manipulated embryos can be transferred either surgically or non-surgically into recipient females in whom the embryos develop into living fetuses and ultimately live young. In fact, no manipulation procedure is considered completely successful until live young have been produced following embryo transfer.

The *in vitro* manipulation of mammalian embryos can be considered under the following general headings, each of which will be discussed in more detail below.

1. Culture — The embryos of a wide variety of mammalian species can now be successfully cultured in defined or semi-defined media.

2. Storage — Many species of embryos can be stored for one to several days at low temperatures (0° to $5^{\circ}C$) without freezing. This storage arrests development of embryos.

3. In Vitro Fertilization — For a few species, it has now become possible to remove unfertilized ova from a female, mix it with semen from the male in vitro, and to produce fertilized ova that will develop into live young when transferred. The most dramatic, and, in many respects, the most successful example of this procedure has been the application to human reproduction, the so-called "test tube baby."

4. Freezing — Over the last 13 years, the embryos of ten mammalian species, including again human, have been successfully frozen to very low subzero temperatures. This permits the embryos to be stored for months, or years, or decades.

5. Micromanipulation — A variety of sophisticated microsurgical techniques have now been applied to embryos. This general procedure can be further divided into four categories.

   a. Embryo Splitting — A single embryo, a genetic entity, can be literally split into two to four pieces, each of which is fully capable of developing into a live young animal. In this way, genetically identical twins, triplets, or quadruplets can be produced.

   b. Chimerism — Two or more individual embryos, or pieces of embryos, can be combined to produce a single animal, a chimera. Several variations of this general concept have been successfully performed. For example, three embryos of the same species but conceived of three sets of parents can be combined. The resultant live young animal has six genetic parents. Alternatively, portions of two embryos at different stages of development can be combined successfully. Although superficially less dramatic, this procedure has important consequences as a research tool. And finally, the most astounding example of chimerism is the combining of embryos or portions thereof from two different species to produce an individual exhibiting genetic characteristics of both species.

c. Gene Injection — During the last several years, true genetic engineering of mammals has become a reality. By this procedure, a gene can be isolated from one species and literally injected into a recently fertilized ovum of a second, totally unrelated species. The resultant transgenic animals, as they have come to be called, will exhibit all of the genetic characteristics of the ovum's species plus that one of the species from whom the gene was isolated. A dramatic example has been the production of "supermice" produced by the injection of multiple copies of the gene controlling growth hormone in rats. The resultant mice produced growth hormone at a much higher rate, and consequently grew faster and larger than normal mice.

d. Nuclear Transplantation — Very recently, Willadsen (1986) has demonstrated that nuclei from 8- and 16-cell embryos can be combined with enucleated halves of unfertilized ova. He cultured such micromanipulated embryos in sheep oviducts, and demonstrated that the embryos were capable of developing into fully expanded blastocysts. Most importantly, when Willadsen transferred such "nuclear-transfer" embryos into recipients, normal live lambs were born. This extremely important research demonstrates that. as Willadsen states, the conditions can be defined "which will allow large-scale cloning of domestic animals."

6. Ovum Maturation — Normally, a mammalian ovum matures within the female's ovary. Upon ovulation, it is fertilized within the oviduct. As mentioned above, _in vitro_ fertilization of ova that have reached maturation within an ovary has become a reality. More recently, it has been shown that immature ova can be taken from the ovary, and caused to undergo maturation _in vitro_. This will have important practical consequences. The female mammal is born with thousands of ova within her ovaries, most of which will not mature and be ovulated. _In vitro_ maturation of ova means that in the future a single female may serve as the genetic mother of hundreds if not thousands of offspring. The genetic consequences of this technique will be somewhat analogous to that of artificial insemination, in which the sperm of a single male can be used to inseminate hundreds or even thousands of females. This latter technique is a common practice in the dairy cattle business.

7. Embryo Sexing — In mammals, the sex of an individual is determined at the moment of fertilization. If the ovum, which always bears an X sex chromosome, is fertilized by a sperm bearing a Y sex chromosome, then the resultant XY embryo developed into a male; if the ovum is fertilized by a sperm bearing an X sex chromosome, then the XX embryo develops

into a female. It has now become possible to determine the sex of an embryo within days after fertilization. This permits sex selection, since embryos of the desired sex can be identified and transferred, embryos of the other sex either being frozen or destroyed. When widely available, this procedure will have important practical application in the cattle industry.

Taken individually or together, these techniques already permit a rather remarkable control to be exerted over reproduction of domestic animals. It can reasonably be predicted that these techniques will eventually be used in wild animals as well. Let us now consider each of the subjects in more detail.

1. Embryo Culture

Probably the single most important technique for in vitro manipulation of mammalian embryos is the capability to culture the embryos. All of the other techniques to be discussed are much more easily studied if treated embryos can be cultured under defined conditions. Species whose embryos have been cultured, at least to some extent, include the following: mouse, rabbit, rat, hamster, pig, sheep, cattle, horse, human. The two species whose culture is most studied and best understood are the mouse and rabbit. Comprehensive reviews of this subject have been published (Anderson, 1977; Biggers et al, 1971; Brinster, 1972; Brackett, 1981; Maurer, 1978; Whittingham, 1971).

Briefly, the purpose of a culture system for embryos is to support the development of the fertilized ovum to the hatched blastocyst stage. All mammalian embryos undergo virtually the same developmental sequence. However, the time required for equivalent development varies between species (Table 1).

Table 1. Rates of Embryonic Development of Different Species

Species	Time (hours) after Fertilization to Morula	 Blastocyst
Mouse	68-77	74-82
Rabbit	60-72	70-144
Pig	72-96	120-144
Sheep	96	144-168
Man	96	120-192
Cow	120	168-192

In most species, the hatched blastocyst implants into the uterine wall of the female shortly after hatching. In a few species, e.g. horse and cow, the embryo continues to expand dramatically after hatching before implantation. Under most circumstances, manipulation of mammalian embryos is performed at the pre-implantation states of development.

In general, the most successful culture system for embryos requires a bicarbonate-buffered salt solution of about 300 mosmoles concentration, a pH in the presence of CO_2 or approximately 7.3, and a temperature of about 37^{o}C. Many variations on this general theme have been introduced. For example, Brinster (see 1972 for review) was the first to demonstrate that mouse embryos require pyruvate and lactate for normal development. Others have studied specific requirements for different species (e.g. Kane and Foote, 1970 for the rabbit; Menezo, 1979 for the cow). At present, the best culture media for the majority of embryos from different species consist of a mixture of salts, amino acids, vitamins, minerals, and a protein source such as fetal calf serum or bovine serum albumin. The majority of media that have been used to culture embryos are now commercially available, although many embryologists insist on preparing their culture media from dry, purified chemicals.

For mouse embryos, 90 to 100% of 2-cell stage embryos or later will develop to the hatched blastocyst stage *in vitro*. It is well established, however, that the rate at which an embryo develops in vitro, even for mouse embryos, does not equal that at which it develops in vivo (Bowman and McLaren, 1970). Nevertheless, under optimum conditions, a high proportion (upwards of 75%) of cultured mouse embryos will develop into live young when transferred into suitably prepared recipients. The transfer of cultured rabbit embryos has been less successful. For example, Maurer (1978) reported that only 11% of 540 rabbit embryos cultured for 72 or 96 hours developed into viable fetuses, whereas 43% of 438 control, non-cultured embryos yielded fetuses. For other species, the results have been highly variable. In cattle, it is generally acknowledged that relatively high pregnancy rates can be obtained with embryos cultured in a variety of media for about 24 hours; longer culture times, regardless of the media, inevitably reduce the number of pregnancies and live calves that result from embryo transfer. Because of the importance of embryo culture to other procedures, research in this area will continue.

Very little is known about the in vitro culture of embryos of wild species. The reason for this can be traced directly to the extreme paucity of such embryos, rather than to any fundamental difference of such embryos. Although there are extreme differences between embryos of different species. it is reasonable to expect that it will be possible to culture embryos of many wild species. But much remains to be learned. Embryos of mouse, rabbit, sheep, and human can be cultured easily. Embryos of rat, horse, and baboon are very difficult to culture successfully. Lessons learned from these species will undoubtedly aid in the culturing of other, more unusual embryos.

Perhaps some additional insight can be gained into alternate preservation methods by studying the natural world. For instance, certain animals, such as the wallaby (Tyndale-Biscoe, 1963) and certain species of bears and bats (Johnson and Everitt, 1984), are known to experience delayed implantation by holding a blastocyst in diapause for up to a year.

2. Embryo Storage

The purpose of embryo culture is to promote the continuing development of the early embryo. Often, the intent is to assess the capability of treated embryos to develop into live fetuses when transferred into recipient animals. However, this may produce a logistical dilemma. Consider the following situation. As described above, a single superovulated cow may yield 20 normal embryos collected from her seven days after she exhibited estrus. To achieve maximum pregnancy, each embryo should be transferred into a recipient that also exhibited estrus seven days previously. This correspondence in estrus is referred to as donor-recipient synchrony. Since the average length of the estrous cycle of a cow is 21 days long, the chance of a given cow exhibiting estrus on the same day as a second one is 1/21. For each embryo to be transferred, then, there must, on the average, be 21 cows available as potential recipients to have one cow in perfect synchrony with the donor cow. For 20 embryos, there would have to be 20 X 21 = 420 potential recipients to have 20 cows in perfect synchrony with the donor. In practice, it has been found that a certain amount of asynchrony can be tolerated without too much effect on the likelihood of pregnancy. Normally, cows are selected as recipients if they have exhibited estrus on the same day as the donor, or one day before or one day after the donor. Therefore, the chance of a given cow exhibiting estrus on any one of three days with respect of the donor's cycle is 3/21 or 1/7. In other words, only 7 cows need to be available as potential recipients for each embryo. For 20 embryos, there would have to be 20 X 7 = 140 potential recipients. However, since only 20 of the 140 cows would actually be used, the other 120 animals would still have to be maintained for future use. The maintenance of a cow not actually being used is a major expense in commercial embryo transfer operations. Any technique that permits a reduction in the number of potential recipients represents a financial saving. Two techniques that are gaining wide-spread use are embryo storage and embryo freezing.

Embryo storage has as its goal the opposite of embryo culture. The latter is designed to promote development, while the former is designed to arrest development, if only temporarily. Attempts to arrest embryonic development by cooling were first attempted more than 30 years ago. Smith (1952) found that almost 70% of fertilized rabbit ova could be cooled to -15°C, if first suspended in a concentrated glycerol solution so that the suspending solution did not freeze. These observations were extended by Sherman and Lin (1958), who found that mouse ova could also be supercooled when suspended in concentrated glycerol solutions. With the advent of embryo freezing, it was quickly recognized that any embryo that was to be frozen would, perforce, have to survive cooling to temperatures of about 0°C. It has now been demonstrated that the embryonic development of mice (Whittingham and Wales, 1969), rabbit (Anderson and Foote, 1975), sheep (Moore and Bilton, 1973), and the cow (Sreenan et al., 1970) can be successfully arrested. "Success" in this context means that live young can be produced from chilled embryos. Recently, Kasai et al. (1983) have demonstrated that mouse embryos can be held at 0°C for as long as three days with relatively little effect, if suspended in concentrated sucrose solutions. Some embryos will even survive for as long as five days at 0°C, although the percentage is low.

One practical consequence of the capability to chill embryos is that is is now possible to ship embryos over long distances in the unfrozen state. The advantage is that chilling of embryos is much easier to perform than freezing. The disadvantage is that embryos can be held at 0°C for only limited times. Two recent examples illustrate the practical value of embryo chilling. It has been found that 60% of more than 350 bovine embryos resulted in pregnancy after transfer, having been shipped at 0°C from San Antonio, Texas to Cody, Wyoming, a trip requiring between 12 and 24 hours (Leibo and Winninger, 1986). Cat embryos can also be shipped at 0°C over long distances without impairing their developmental capability (Leibo and Dresser, unpublished observations).

This technique will permit embryos as experimental material to be exchanged between laboratories.

There is one final, important note regarding embryo chilling. As far as is known, pig embryos will not survive if cooled below about 10°C, regardless of the suspending solution, the cooling rate, or the warming rate (Polge, 1977).

3. In Vitro Fertilization (IVF)

The capability to fertilize mammalian ova *in vitro* is a technique of enormous fundamental and practical importance. First, it permits the detailed study of the whole complex series of events of fertilization. Second, it offers the potential to drastically increase the number of offspring that a given female may produce. It can also dramatically increase the reproductive lifetime of a female, since ova for IVF can be obtained prior to sexual maturity and can be obtained long after a given female is able to conceive naturally. Since numerous reviews and books devoted to this subject have been published recently (Hafez and Semm, 1982; Mastroianni and Biggers, 1981; Brackett, 1983), this subject will be discussed only briefly here. With the explosive growth of IVF in human clinical medicine, there is now a journal (*Journal of In Vitro Fertilization and Embryo Transfer*) explicitly devoted to this subject.

Evidence has been published that some steps of IVF have been accomplished in almost 20 mammalian species. However, true success, in the sense that live young have been born, has been documented only for the mouse (Whittingham, 1968), rat (Toyoda and Chang, 1974), rabbit (Brackett and Oliphant, 1975), human (Steptoe and Edwards, 1978), cow (Brackett et al., 1982), baboon (Clayton and Kuehl, 1983; Bavister et al., 1983) and monkey (Balmaceda et al., 1986). Briefly, there are several reasons why IVF has proven to be such an intractable problem for such a long time. First, practical methods to recover mature ova from the ovary must be developed. In laboratory species such as the mouse and rabbit, this is relatively easy. In large domestic animals such as the cow and horse, this has been much more difficult. Recent progress in the refinement of instruments for laparoscopy have substantially improved this situation. Interestingly, the impetus was provided by the rapid development of IVF procedures in the human. It is also ironic that success in human IVF actually preceded that in non-human primates. Often, justification for research in these latter species is stated to be as models for human research. Once mature ova have been collected from a female, conditions to maintain these ova must be defined. The problem is that fertilization is a concatenation of events. Even though optimum conditions for certain steps may be used, the entire process may fail if only one step is performed incorrectly. For example, one extremely important step is capacitation of sperm. For some species, conditions under which sperm can be rendered capable of fertilizing ova have been defined. Even when conditions for ova maturation and sperm capacitation have been defined, many problems still remain. Basically, these again relate to embryo culture. It has long been recognized that the metabolic requirements of different stages of embryos are different. When the embryo changes from maternal to zygotic control of development, its metabolic requirements change. The stage at which this change occurs varies for different species. Attempting to define the optimum conditions for the various embryonic stages of different species is a difficult problem. Given the fact that these conditions have been defined, at least to a limited extent, for seven species certainly does suggest that progress in this area will be forthcoming.

At present, and with the notable exception of the human, IVF of mammalian species is still largely an experimental technique. However, recent continuing progress with IVF of bovine ova strongly suggests that this procedure will shortly move from the laboratory to the clinic (First et al., 1985). How quickly IVF can be accomplished with other domestic animals will obviously depend on the extent of research support for this area.

In vitro fertilization has not yet "come to age" in wild animals; that is, it has had very little attention thusfar as a technology which can be adopted to improve the reproductive potential of wild animals. Most of the work that has been done on these animals has been on non-human laboratory primates for the purpose of human-oriented research such as to observe any abnormalities which may be related to laboratory conception, or to help differentiate genetic versus maternal influences on fetal growth and development.

In vitro fertilization could have tremendous potential in wild animals. The collection of eggs which is required for *in vitro* fertilization is the female equivalent of semen collection, and many of the same advantages and disadvantages apply. *In vitro* fertilization has a long way to go in its application to wildlife, but once the techniques of ova collection, ova preservation, and the actual fertilization of an ovum by sperm are worked out, the applications are overwhelming. Squirrel monkey ova have already been successfully frozen and thawed (De Mayo and Dukelow, 1983). If eggs can be "banked" like semen, it will be possible to produce a living embryo from frozen germ cells without the actual presence of either of the parent animals who have contributed their germplasm. This will contribute substantially to maintenance of the genetic diversity of a captive gene pool.

4. Embryo Freezing

The first successful freezing of mammalian embryos was reported in 1972 (Whittingham et al; Wilmut). Since those reports, more than 300 articles and 100 abstracts have been published on this one subject. Three full meetings (Ciba Foundation Symposium 52, 1977; Muhlbock, 1976; Zeilmaker, 1981) and numerous symposia devoted exclusively to the freezing of mammalian embryos have been convened. Numerous reviews have also been published (Lehn-Jensen, 1981; Leibo, 1977, 1981; Maurer, 1978; Rall et al., 1982; Renard, 1982).

Freezing of mouse, rabbit, and bovine embryos has now become a routine procedure. All together, embryos of eleven mammalian species have now been successfully frozen. Again, "success" means that live young have been born from frozen embryos. To date, the species that have been successfully preserved include the mouse (Whittingham et al., 1972), cow (Wilmut and Rowson, 1973), rabbit (Bank and Maurer, 1974), sheep (Willadsen et al., 1974), rat (Whittingham, 1975), goat (Bilton and Moore, 1976), human (Trounson and Mohr, 1983), horse (Yamamoto et al., 1982), baboon (Pope et al., 1984) and antelope (Kramer et al, 1984). Only several weeks prior to the publication of this manuscript, two litters of kittens were produced from frozen-thawed cat embryos by Dresser et al. The freezing of mouse embryos has become so routine that banks of tens of hundreds of thousands of mouse embryos have been frozen to preserve valuable genetic stocks for extended times. For some species, most notably mouse, rabbit, and cow, preservation by freezing has reached a level of sophistication and reliability so that approximately 80 to 90% of frozen embryos will develop in vitro when thawed and cultured. Moreover, the procedures to freeze embryos have become increasingly simplified. Recently, for example, methods to freeze embryos by techniques that require minutes to perform, rather than the one to two hours previously required, have been published. Renard et al. (1984) have reported that high survival of rabbit embryos can be obtained when they are frozen in a solution of propanediol plus sucrose. The samples are cooled to -30°C, held briefly, and then plunged directly into liquid nitrogen (LN_2) at -196°C. They also found that approximately the same percentage of frozen-thawed embryos as control, unfrozen embryos would develop into live young when transferred. Massip and van der Zwalmen (1984) have reported similar observations for bovine embryos frozen in glycerol plus sucrose. Very recently, Rall and Fahy (1985) have found that approximately 90% of mouse embryos will survive when plunged directly from 0°C to -196°C after having been suspended in a complex solution of dimethyl sulfoxide, acetamide, propylene glycol, and polyethylene glycol. The novel feature of this solution is that it does not freeze, but rather vitrifies. That is, the solution does not crystallize but becomes glass-like when cooled to low temperatures. Rall and his colleagues have now found that approximately 30% of vitrified embryos will develop into live fetuses when transferred into recipient foster mothers.

In addition to these novel methods of preserving mammalian embryos, other research has been devoted to simplifying other aspects of embryo freezing. Much of this work has concentrated on the freezing of bovine embryos as an important adjunct to the commercial embryo transfer industry. Several reports have been published recently that describe methods by which bovine embryos can be frozen in such a way that they can be thawed under field conditions without the use of a microscope or any other laboratory apparatus. The advantage of these methods is that it is now possible to perform transfer of bovine embryos in a fashion analogous to artificial insemination. Independently, Renard et al (1982) and Leibo et al. (1982, 1983, 1984) have found that high survival of bovine embryos can be obtained when they are frozen in glycerol within plastic insemination straws together with sucrose as a diluent. In practice, a thawed embryo can be mixed with the sucrose within the straw in which the embryo was frozen. This means that the time consuming stepwise dilution of frozen-thawed embryos normally performed with a microscope can now be carried out in minutes without any special apparatus at all. Recent experience with this technique in a commercial embryo transfer company illustrates its practical value. A total of 1078 embryos collected from 173 donor cows were frozen by this One-Step method, as it has been called. The embryos were thawed and transferred within about five minutes after thawing. These transfers yielded a total of 425 pregnancies, or 39% pregnancy. Of equal importance was the fact that an average of 2.5 pregnancies per donor cow were produced. The reason to stress this fact is that higher percentages of pregnancy have occasionally been reported. Often, however, that results from the fact that only the best quality embryos have been frozen, and any embryos that have obviously degenerated after thawing are not transferred. The effect is to increase the percentage pregnancy, although this reduces the number of pregnancies per donor whose embryos were frozen.

Regardless of the methods used, freezing of embryos of domestic species is not very widely practiced. This is particularly true for bovine and sheep transfers. Probably the major impediment to the more widespread use of freezing in the equine embryo transfer business is simply the lack of experience. Nevertheless, it can be reasonably estimated that thousands, if not tens of thousands, of live young of domestic species have been produced from frozen-thawed embryos.

Before leaving this subject, it must be noted that the freezing of mammalian embryos rests on a firm mechanistic understanding of the factors responsible for cell injury caused by freezing, thawing, and dilution. These mechanisms have been reviewed recently (Leibo, 1981, 1985; Mazur et al., 1984; Schneider and Mazur, 1984). Because of this understanding, and given the impetus both of the commercial bovine embryo transfer industry and of human IVF practice, it is reasonable to predict that even more progress will be made in this area in the future.

Again, because of extremely limited experimental material, only a few attempts have been made to transfer previously frozen wild animal embryos into recipients. The procedure has yielded some limited success (Cherfas, 1984).

Cryopreservation will undoubtedly prove to be an important adjunct to reproductive research in non-domestic animals. Geneticists Thomas Foose and Ulysses Seal have determined that a population of 250 properly managed animals of a particular species can theoretically preserve 95% of the original genetic diversity of the group after 50 generations (400 years) (Myers, 1984). The world's zoos, however, have limited facilities, and often cannot accommodate large numbers of animals of each species they maintain. Success in the area of cryopreservation will allow zoo professionals to overcome the limited space in zoos and wild animal preserves by maintaining the bulk of the desired genetic diversity in liquid nitrogen freezers. Embryos containing new bloodlines could be recovered in the wild, and brought back to zoos in the frozen state to improve the bloodlines of captive populations without depleting the wild herds. U.S. government restrictions currently prohibit the importation of embryos from other countries, but scientists have been actively lobbying for a change in these restrictions; it appears that a change may be possible in the future.

5. Embryo Micromanipulation

This is an area that has witnessed dramatic progress in little over six years, especially as it applies to domestic animals. The research of Willadsen (1979, 1980, 1981) is most notable in this regard. Important contributions have also been made by Ozil et al. (1982), Heyman et al. (1985), and Williams et al. (1982).

One example of micromanipulation is the technique by which an embryo is microsurgically dissected into two or more pieces. It had previously been shown that the individual blastomeres, or cells, of 2-cell or 4-cell stage mouse embryos could be separated, and that each cell was capable of developing into a normal living animal when transferred. In 1979, Willadsen demonstrated that similar procedures could be performed with sheep, goat, horse and bovine embryos. By separating the blastomeres, and culturing them *in vivo* within a ligated sheep oviduct, he was able to produce large numbers of genetically identical twins, triplets, and even quadruplets. As remarkable as these observations were, they were of relatively small practical value in the commercial embryo transfer field because Willadsen had used early cleavage-stage embryos. Almost all commercial embryo transfer practitioners collect bovine and equine embryos non-surgically. With this technique, one is limited to the collection of six to eight day embryos, e.g., morulae or blastocysts. It is only at this stage that the embryos have passed through the oviduct into the uterus, where they are accessible to non-surgical collection. Williams et al. (1982) demonstrated that Willadsen's basic technique could be adapted to late stage embryos. By literally splitting bovine embryos into two pieces with a small razor blade chip, they were able to produce genetically identical twins. Recently, other workers have repeated these observations. For example, Lambeth et al. (1983) bisected 18 bovine embryos, and produced 11 live fetuses. More recently, the techniques have been adapted to commercial use. Baker and Shea (1985) have reported on the use of this technique in a commercial context. Experience in a commercial facility illustrates the practical value (Leibo, 1986, unpublished observations). A total of 275 embryos were split and transferred as half or demi-embryos. If those embryos had been transferred intact, then one would have expected to produce between 165 and 180 pregnancies (60 to 65% pregnancy). Splitting the embryos to produce 550 demi-embryos yielded 291 pregnancies. Although the percentage pregnancy was reduced (291/550 = 53%), at least 111 more pregnancies resulted than would have by standard intact embryo transfer. This amounted to a 65 to 75% increase in pregnancy production. There can be no question that this procedure is finding wide use at a commercial level.

An even more dramatic result of micromanipulation has been the production of chimeras. These are animals which have two or even three sets of genetic parents. This is accomplished by combining two or even three embryos at a relatively early stage of development. One dramatic example of this was the "manufacture" of hexaparental mice by Markert and Petters (1978). These tricolored mice carried genetic characteristics of all three sets of parents. The construction of chimeric embryos is perhaps the most promising technique for overcoming the rejection mechanism of the surrogate's placenta in an interspecies embryo transfer. By micromanipulation it is possible to prevent the surrogate uterus from "recognizing" the foreign embryo by combining chronologically different blastomeres. The younger cells tend to form the trophoblast that gives rise to the placenta, and the older cells tend to form the inner cell mass that will form the fetus. Chimeric embryos can be constructed so that the cells which comprise the trophoblast belong to the surrogate species and the cells which comprise the inner cell mass belong to the donor species. A "reupholstered" embryo such as this can effectively trick the surrogate's uterus into thinking it is carrying a native embryo (Vietmeyer, 1984).

Interspecies embryo transfer and inner cell mass transfer should not be confused with hybridization of species. Hybridizing species has not been a popular approach to preserving wild species, although successful births have resulted from tiger/lion and several antelope species (Fifield and Singh, 1985) when crossed. Hybrids contain mixed genetic material from two different species whereas offspring produced from embryo transfer or inner cell mass transfer contain only genetic material of the original species. This technique has now been adapted to domestic species with astonishing results. Recently, Fehilly et al. (1984) and Meinecke-Tillmann and Meinecke (1984) have independently reported the construction of chimeras of sheep and goat embryos. The live young (especially those of Fehilly et al., 1984) clearly carry the phenotypes of both species. This technique will have important fundamental and practical consequences, since it is the one demonstrated method by which species barrier to reproduction can be circumvented. Many writers have now recognized that this method might be utilized to "rescue" endangered species. It might be possible to construct chimeras consisting of an embryo from an endangered species plus an embryo of a common, but related species. The common species might then carry the fetus of the endangered species to term.

One final example of micromanipulation certainly deserves mention. This area of research represents true genetic engineering of mammals. This is the production of transgenic animals by the microinjection of exogenous DNA into fertilized ova. In work that was first reported only four years ago, it was demonstrated that a gene isolated from one species could be successfully transferred into a fertilized ovum of a second species. When such injected ova were cultured and then transferred into recipients, a portion of the offspring expressed the foreign gene (Gordon and Ruddle, 1981; Wagner et al., 1981). Since those first reports, there have been many more similar examples. One of the most dramatic was the demonstration that the gene complex that codes for metallothionein-human growth hormone was capable of stimulating the growth of mice (Palmiter et al., 1983). This followed from the earlier work of the same group who demonstrated the expression of rat growth-hormone gene in mice. What was particularly striking about their work with the metallothionein gene was that the activity of the growth hormone gene could be induced by administering cadmium or zinc to the mice. In other words, they were able to control the expression of the foreign gene. Very recently, this same group has successfully produced transgenic rabbits, sheep, and pigs by microinjection of foreign DNA (Hammer et al., 1985). Again, they were able to exert control over the expression of the "new" gene. What has been extremely important about these observations is that they represent a marriage of molecular biology and mammalian embryology. As these observations are extended, as they certainly will be, greater understanding of the molecular biology and genetics of mammalian embryology will emerge. At the moment, of course, these sophisticated techniques of genetic engineering of mammals are very much laboratory *tours de force*. There can be little question that the production of transgenic farm animals will quickly find application in commercial activities.

One final method of micromanipulation should be mentioned. Nuclear transfer offers the potential of true cloning of mammals. By this method, nuclei removed from the cells of late-stage embryos are transferred individually into fertilized ova. Each resultant embryo is genetically identical, as are the live animals that result.

The most useful method for cloning desirable individuals of a species would be the substitution of nuclei from the somatic cells of desirable individuals for nuclei of one-cell embryos. Although this technique is almost certainly not possible in mammals, it has been the subject of a great deal of fanciful speculation regarding the regeneration of intact mammals from long-frozen tissues of extinct species such as the woolly mammoth (Daniel, 1981). In anticipation of future breakthroughs in technology, the San Diego Zoo currently maintains a repository of fibroblast cells taken from the connective tissue of hundreds of exotic animals (mostly kidney, spleen, and liver cells) (Benirschke, 1984). These cells, which are easier to store and grow than most, have been frozen with the hope that the technology of somatic cell cloning might become available. Scientists are urging each other to begin collecting and storing tissue samples from rare animals for future use whenever possible. According to Dr. Kurt Benirschke, "You must collect things for reasons we don't yet understand." (Benirschke, 1984).

ARTIFICIAL INSEMINATION

Artificial insemination is the introduction of semen into the vagina, cervix or uterus by artifical means. Artificial insemination was supposedly used by the Arabs in ancient times, but the first documented success using artificial insemination in the modern world was in 1794 with a dog (Betteridge, 1981). Artificial insemination of livestock was used extensively in Russia by the 1930's. Arthur Walton demonstrated its potential as an effective method to transport genes in the 1920's and 1930's by shipping fresh rabbit, sheep, and bulls semen from England to other European countries (Betteridge, 1981). The ability to successfully freeze semen resulted from the accidental discovery of the cryoprotective action of glycerol by Polge, Smith and Parkes in 1949 (Betteridge, 1981). Artificial insemination is very common in the agricultural industry today. In 1981, Foote estimated that close to 90 million head of cattle were produced through artificial insemination worldwide in 1977 using frozen semen. Artificial insemination has had limited success in wild animals thusfar, especially with certain species of mammals and birds, including the rare whooping crane and giant panda (see Appendix II). However, much more semen has been collected than has actually been used (Howard et al., 1984 a and b; Wildt et al., 1983). The first successful artificial insemination of a wild species using previously frozen semen occurred in 1973 by Dr. Stephen Seager of Texas A and M University with the wolf (Seager, 1981). This was followed by the successful insemination of a gorilla (Douglass and Gould, 1981). There has been a great deal of time and effort spent trying to artificially inseminate wild caught felidae, almost all of which has resulted in failure (Dresser et al., 1982a). The London Zoo finally produced a puma in 1980 through surgical artificial insemination using a fresh semen sample (Moore et al., 1981). This was followed by a successful nonsurgical artificial insemination of fresh semen in a Persian leopard at the Cincinnati Zoo in 1981 (Dresser et al., 1982a).

There are three methods of semen collection: manual stimulation of the male reproductive tract, use of an artificial vagina, and electroejaculation (Salisbury et al., 1978a). Electroejaculation was invented by two French workers, Jondet and Cassou, and is by far the most common collection mode for wild animals. The use of electroejaculation and artificial vagina have been applied to semen collection in over 100 species of zoo animals (Seager et al., 1980). Electroejaculation works by inserting a lubricated probe into the rectum of an anesthetized animal which conveys mild pulsating electrical stimuli to the nerves of the reproductive tract, resulting in ejaculation. There is some question about the fertility of sperm collected through electroejaculation, but there is also a question about how viable a semen sample must be to be effective. For example, the successful gorilla insemination at the Memphis Zoo in 1981 was done using a previously frozen sample whose motility was 10% and judged "poor" at the time of insemination (Douglass and Gould, 1981).

There are a great many problems associated with artificial insemination as it applies to wild animals. First of all, it necessitates the use of anesthesia, which is always a risk, for both semen collection from the male and insemination of the female. The fertility of semen obtained from artificial collection techniques is sometimes questionable when compared to that produced in a natural ejaculation (Salisbury et al., 1978a). Sperm usually begin to die as soon as they are collected. Even if a fresh sample is used for insemination, it is likely to have undergone a certain amount of sperm loss. The freezing process is also somewhat detrimental to sperm, and it is very unlikely for sperm to be as motile after being frozen (Cochran et al., 1985). Much work needs to be done in the area of semen cryopreservation. To date, sperm has been frozen from at least 200 different species, but very little of it has actually been thawed and tested (Seager, 1981). The ultimate test is production of offspring. Cryobiologists have found, from semen that has been tested, that sperm from each species needs to be extended and frozen under slightly different conditions to produce the optimal results (Graham et al., 1978; Platz et al., 1978; Iritani, 1980; Pickett et al., 1976).

Semen extender basically consists of a buffered solution that contains a cryoprotectant (e.g., glycerol), antibiotics, and either egg yolk or milk. There are many variations which have been tried in the basic recipe for semen extender. The agricultural industry has found that the optimal extender for a given species seems to be very species-specific, and that is turning out to be true with wild animals also. Perhaps some clues for semen preservation can be found in the natural world from studying certain female reptiles who have the potential for keeping sperm viable within their reproductive tracts for up to six years after mating (Howarth, 1974).

Other problems with artificial insemination include difficulty in predicting the optimal time for inseminating the female and the fact that artificial insemination cannot occur as frequently as a female would have been inseminated naturally for the duration of her estrous cycle (Salisbury et al., 1978b).

On the positive side, artificial insemination can be a great boon toward improving the genetic diversity of a captive population of animals. The risk and expense of transporting semen is far less than that of transporting a male animal for breeding purposes. Artificial insemination could also be used to overcome quarantine restrictions and the risks of disease. Often, the strict agricultural legislation has made transport of zoo animals more difficult and costly than is perhaps necessary. Although some disease viruses could be transported in semen samples, the importation of semen rather than whole animals has been hailed as one way to avoid spreading pathogens (Cherfas, 1984).

PROPOSAL TO IMPROVE TECHNOLOGIES

A. Domestic Species

As described above, a wide variety of procedures have been developed to control reproduction in domestic animals. Many of these techniques have evolved gradually over a long period of time, while others have reached a level of commercial applicability relatively rapidly. As is true in all of science, numerous investigators have contributed to the development of these disciplines. Many of the contributors to these disciplines have conducted their studies in the traditional centers of fundamental research, namely, in the academic community and in public, non-profit research institutions. But many others have performed equally important research in commercial organizations. The reason in the case of mammalian embryo transfer is rather obvious. The last ten years, and, even more, the last five years, have witnessed an extremely rapid growth in commercial embryo transfer (ET). According to a recent newsletter of the International Embryo Transfer Society (IETS), there are now more than 115 commercial ET companies, and another 100 veterinary practices that offer transfer services. A survey conducted by the IETS indicated that at least 165,000 bovine embryos were transferred internationally during 1983. Assuming a 60% rate of success, this means that close to 100,000 bovine pregnancies would have been produced. All indications are that these figures have continued to increase. Because of the rapid changes in ET technologies over the past five to ten years, many commercial companies have decided that it is in their commercial self-interest to establish research capabilities. At least six ET companies have built research laboratories, modest though they may be. Investigators in these commercial laboratories publish scientific papers, and participate actively in the scientific community. Another measure of the impact of bovine embryo transfer on research in this area is that almost 40% of 220 papers published between 1977 and 1983 that relate to embryo freezing dealt specifically with bovine embryos.

Taken together, all of this means that the techniques of embryo transfer are under active investigation. There is no indication that there will be any abatement in the intensity of research in the near future. That is not to say that there is no room for improvement, or no need for further research.

The biggest single variable in the entire procedure of ET is the response of donor females to superovulatory hormones. This is particularly true of cattle and horses. Apparently, the mare is almost completely unresponsive to the induction of superovulation. And the cow responds in a highly variable fashion. If it were possible to induce superovulation in a more reproductible way, then the entire efficiency of ET would be improved. If this problem were to be solved, of course, then embryo transfer technologies might actually be used in a way that would appear to reduce, rather than to maintain, biological diversity. The reasons are discussed in the following paragraphs.

At the present time in the U.S. cattle industry, certain breeds and even individual animals are more popular than others. The breeders of purebred cattle are constantly seeking ways to enhance the attraction of their breed, or their donor cows. It is analogous to the introdution of new models by the automobile industry, or new hybrids by flower growers. The new model or hybrid may not be better than the old, but it is different. That attracts buyers. As a consequence, donor cows are selected for an ET program because the owner hopes that calves from a given cow will be more popular than others, and will sell for higher prices. In other words, donor cows may not necessarily be selected because they are genetically superior. And even those donors that are truly superior can exert only a very minor genetic impact, since the average donor yields only about 20 to 30 calves per year. If superovulation techniques were to be improved, and if some of the other techniques described above were to be used on a wide scale, then it might become possible for a single donor cow to produce 200, 300, or even 1000 calves in her lifetime. Consider the following. (1) Assume that superovulation induced the maturation and ovulation of 50 ova per collection, rather than the present 10 to 15. (2) Assume that in vitro fertilization could be performed reliably and with high efficiency. (3) And finally, assume that cloning of 4 or 8-cell stage embryos has become an established procedure. The following situation might then become possible. Six collections of ova from one donor per year would yield 300 ova. If in vitro fertilization were 80% efficient, then 240 embryos would result. These could be cultured to the 4-cell stage, and each embryo could be cloned to produce four new embryos. Thus, a total of 960 embryos might be available from a single donor in one year. Freezing, of course, would be used to bank embryos, much as semen from a genetically superior bull is now stored in the frozen state. This proposition does not even presuppose any use of genetic engineering, embryo sexing, or ovarian ova maturation. This scenario would seem to offer the prospect of less, rather than more, biological diversity. We believe that the opposite would actually be true.

If any of the assumptions above prove to be even approximately valid, then it will become possible to "manufacture" domestic animals to perform specific functions. The genetic impact of a single cow capable of producing 1000 offspring would be large. But genetic diversity could, in a sense, be maintained much more reliably by direct control of the genetics of a single animal. Finally, of course, embryo freezing almost certainly will continue to be made more reliable and simpler to perform.

One obvious option available at the present time to maintain biological diversity would be to begin to bank representative genotypes of domestic animals in the frozen state. Once an embryo is frozen to -196°C, it can almost certainly be maintained in a state of suspended animation for hundreds if not thousands of years. The cost of maintaining an embryo in the frozen state is miniscule. There are several impediments to the establishment of a bank of domestic animal genotypes. The first is that the embryos of a donor animal are considered to be the property of the donor's owner. There is, at present, no advantage to an owner to contribute some of his frozen embryos to a domestic animal embryo bank. Another impediment is that there is simply no common place where frozen embryos of domestic species can be banked.

One final point needs to be made with respect to the techniques described above. There is clearly need for further research in all of these areas. Although perhaps modest at the present time, financial support of research dealing with embryo transfer technologies by the private sector is clearly increasing. Many state academic institutions also provide generous financial support of ET research. Notable among these are Colorado State University, Texas A and M University,, Louisiana State University, Florida State University, the University of California at Davis, Ohio University, the University of Wisconsin, and the University of Minnesota. Research at private universities, funded by the federal govenment, tends to concentrate on laboratory species. This is extremely important, since research on laboratory species has often served as models for domestic animals. There are, of course, limitations to this approach. Often, there is no substitute for the domestic animal itself. Unfortunately, domestic animal research is extremely expensive.

B. Wild Species

Much action needs to be taken to promote research and development of the above discussed technologies. With wild species, little is known about basic reproductive processes. Knowledge of these processes is a prerequisite for the development of technologies that will maintain biological diversity in animals. Action on the part of interested institutions and scientists rather than discussion and lengthy plans to develop and refine technologies would help to increase the rates of application and success of technology. Not that theory and plans are not important, but too often this is all they become.

As discussed above, very little is known about the reproductive cycles of most species of wild animals. Advanced reproductive technologies are available from prior research accomplished and proven in some species of domestic animals. These technologies need to be tried and repeated in wild animals so that their genetic material can also be preserved. United States scientists and government should consider the privilege it is to have animals from all other continents in the world available for study, and should commit time and money to understanding the knowledge these species can provide. More field studies in the native habitats of these animals should be promoted so that comparisons can be made between wild animals and those in captivity. Normal physiological and behavioral values would then be available as a foundation for study.

Since preservation of genetic material from a species is one of the key answers to insuring their diversity, as far as technology development is concerned, emphasis should be placed on gamete preservation, most probably cryopreservation. Cryobiology needs to be recognized as a scientific discipline so that professional programs will be outlined by universities and more students will concentrate their studies in this area. With its recognition as a scientific discipline, more federal granting agencies might award more funds for cryobiological research. This expertise could then be applied to the preservation of wild animal germplasm. The more that is understood and developed with domestic animal germplasm, the more chance there is to be successful with germplasm from wild animals.

Zoos and curators of zoological collections should be made aware of available technologies, as should other governments that hold the tremendous amount of wealth in biological diversity in their animals. They should be informed as to the benefits and risks of using these technologies. Common and more numerous relatives of many wild species should be targeted for technology development, and then action taken to move forward with these species until these technologies are successful. Many animal breeds, strains and species representatives should be put "on ice" before their numbers are drastically reduced for whatever reason (disease, warfare, food source, habitat destruction, poaching, etc.).

In theory it is simple: basic knowledge should be gathered on animal reproductive processes, technology should be developed, applied and refined and animal germplasm should be preserved. However, while theory is being placed on volumes of paper, animals and plants are being lost. Decisions should be made more quickly and action taken. If, as experts predict, one species of plant or animal will become extinct each day by 1990, biological diversity will be diluted at an alarming rate. There is much we have not learned. Yet, we have the opportunity to learn much. If already interested and concerned scientists are given additional incentive, such as support from the U.S. government, to pursue their interests, animal diversity may more quickly be maintained. Biological research should be financially supported at the laboratory level so that technology for animal germplasm preservation advances at a quicker rate — a rate at least closer to the extinction rate.

PROPOSED INSTITUTIONAL AND CONGRESSIONAL ACTIONS

The disciplines described above have been changing at an extremely rapid rate. As a consequence, there is an ever-increasing need for additional funding to support research in these areas. Financial support to study many aspects of domestic animal reproduction has been provided to both the public and private sector. Until rather recently, however, the perception of many in the scientific community has been that a "natural" source of funds for research on animal reproduction, namely, the U.S. Department of Agriculture, has been quite modest in its support. That is, research to study various aspects of domestic animal embryology has not been perceived as being vigorously supported by the U.S.D.A. This stands in contrast to the situation that is perceived with respect to research in these areas in other countries. For example, for several years the Canadian Department of Agriculture has supported extensive research to determine whether viruses and bacteria are transmitted from infected donor animals to recipients. Fortunately, it appears as if embryo transfer is an excellent method to prevent disease transmission as a result of animal reproduction. Nevertheless, the Canadian program has been very "visible" to the scientific community. Even if the U.S.D.A. has provided support for this research, it has not been nearly so well recognized. Another example is found in a very well suppported program on bovine embryology in France. The French equivalent of the U.S.D.A., I.N.R.A., has for many years provided support for five laboratories studying bovine embryo culture, freezing, micromanipulation, and sexing. What is even more remarkable about the French program is that INRA laboratories apparently work in collaboration with a French commercial company, Instruments Medicale Veterinaire. IMV operates all over the world selling devices and instruments to perform artificial insemination of domestic animals. More recently, IMV has begun to market instruments for embryo transfer. Apparently, instruments and procedures developed in INRA laboratories are commercially marketed to the international cattle industry by IMV. This collaboration makes formidable competition for small U.S. companies who would like to operate in international markets.

Fortunately, it appears as if the U.S.D.A. attitude toward embryo transfer and related disciplines has recently changed. Two examples illustate this change. The U.S.D.A. has begun to collaborate with the American Embryo Transfer Association regarding regulations for the import and export of frozen embryos. The perception of the U.S.D.A. in this area by workers in the field is very positive. Apparently, U.S.D.A. officials together with the Department of Commerce have been assisting the A.E.T.A. in formulating regulations and guidelines. An even more striking example of the U.S.D.A.'s changed attitude toward embryo research is found in the studies described above regarding transgenic rabbits, sheep, and pigs. Investigators at the U.S.D.A.'s Beltsville laboratory have played a prominent role in that research.

Given that changed attitude of the U.S.D.A., and given the concern that new technologies may decrease biological diversity, it seems reasonable to suggest a new responsibility for the U.S.D.A. As mentioned above, one option to maintain biological diversity that is immediately available would be to establish a bank of animal germplasm. The cost to keep embryos frozen is really quite modest. Once frozen, however, embryos remain viable for years. It has been formally estimated from studies of radiation biology and physical chemistry that frozen embryos will probably remain viable for hundreds to thousands of years (see Leibo, 1986).

We suggest that the private sector (owners of donor cows and ET companies) could collaborate with the U.S.D.A. by "depositing" frozen embryos of domestic species in this embryo bank. A panel of university investigators could decide on what "genetics" ought to be deposited in the bank. A modest tax incentive could be offered to the private sector to encourage it to contribute to the bank. Since the cost to establish and maintain such a bank should be extremely small (the Jackson Laboratory mouse embryo bank could be used to provide a reliable estimate of the costs), the U.S. Congress ought to be able to demonstrate genuine altruism by the immediate authorization of a U.S.D.A.-funded Bank of Animal Germplasm.

As far as wild animal germplasm is concerned, government support, the National Institutes of Health for example, will be essential so that several zoological institutions nationwide could concentrate on the development of technologies for germplasm preservation. Zoos and wild animal preserves are the greatest source of animals for this effort. Therefore, federal granting agencies should appropriate more funds toward these, usually non-profit institutions, and designate several zoological institutions that are geographically dispersed as research centers to concentrate on reproductive research and techology development.

Empirical study of animal reproduction processes will not be sufficient to further develop technologies for the preservation of wild animal germplasm. These technologies and the urgency of their development are far too important. Too often, in the case of wild animals, initial techniques are developed for one species and then assumed to suffice for related species. In most situations, what works for one species, does not work for another. For example, some semen extenders and freezing procedures that are successful for the preservation of tiger semen are not adequate for leopard semen. Leopard sperm motility will decrease rapidly following collection and, unless immediately used for artificial insemination, will be lost (Dresser et al., 1982a). Post-thaw survival of sperm following cryopreservation is not the same for all species, even when they are frozen and thawed by exactly the same procedures. Such is the case also for embryos. Some mammalian embryos (e.g., pig) have never been successfully frozen and thawed, even though techniques have been used that have already proven successful with embryos from other species. Today, no one can overestimate the importance of freezing cattle embryos. Yet it took 25 years from the time the theory was presented and live offspring were produced. This cannot happen with many wild species or the species will already have become extinct.

The freezing of animal sperm (Appendix II) and embryos has now proven successful as live offspring have been produced. Embryos from 11 mammalian species have been successfully preserved, and thousands of live young have been produced in several species (e.g., mouse, rabbit, cattle) from frozen-thawed embryos. To date, there have been no observations of teratogenic effects (birth defects) as a result of cryopreservation.

Since empirical studies do not seem to suffice and greater concentration needs to be placed on development of technology for each different species, it is recommended that more government funding be directed toward postdoctoral fellowships in wild animal germplasm preservation. There needs to be serious concentration on all of the individual problems and questions facing this area of research. Scientific manpower is greatly needed. It is also recommended that a national plan coordinating technology development be prepared by a panel of scientists and government officials and joined together with the ongoing Species Survival Plan of the AAZPA (AAZPA, 1983) for species that have already been selected as significant due to their decreasing numbers both in the wild and in captivity. A plan of technology development of this sort would help to guide reproductive scientists as to order of importance for certain technologies.

Another recommendation for the preservation of wild animal germplasm follows the progress of technology development. Once technologies have been proven successful for common relatives of rare species such as for antelope (Dresser et al., 1984b, 1985), the movement of animal germplasm across continents should be approved. To maintain biological diversity of animals, germplasm should be collected from animals still in a wild situation, preserved, then "banked" in approved laboratories that are financially maintained by the United States government. The United States Department of Agriculture should promptly consider the potential of transporting germplasm from animals worldwide, and regulations should be established to make this possible. There are many European breeds of domestic animals that should also be imported in an embryonic state and preserved in germplasm banks.

Finally, it is recommended to the U.S. Congress that a National Repository for Animal Germplasm be established so that the management of technology development and the maintenance of preserved germplasm can be coordinated and insured indefinitely. There now exists the unique opportunity to preserve biological material from domestic and wild animal species. It is an opportunity that should be given high priority.

It is suggested that this National Repository be for long term storage of germplasm but not the only place germplasm be actually frozen. Other academic and qualified institutions and commercial organizations could continue technology development and then contribute germplasm to this National Repository on a voluntary or tithing basis. Since there already exists a National Seed Storage Laboratory in Colorado for plants supported by federal funds, and a mouse embryo storage bank (Jackson Laboratories) supported by the National Institutes of Health, the government should now consider the importance of the preservation of biological diversity by establishing a "bank" of germplasm from unusual species. This is an option that is presently available, but this option may disappear before this century is over. The zoo "ark" may not remain large enough if some animal species are not condensed to embryo size!

All mammalian species might be included in this National Repository. Scientists could be asked to contribute germplasm, and their institutions would become members. There could be a scientific advisory panel that would represent academic, commercial and zoological institutions. If biological diversity is to be maintained, some action must be initiated promptly.

SUMMARY

The last ten years has witnessed an enormous growth in the capability to control reproduction in animals. One potential danger of the new technologies is that there will be a loss of genetic diversity as animal reproduction becomes more and more controlled.

Among the technologies that are now used in conjunction with embryo transfer to control animal reproduction are a variety of techniques to manipulate mammalian embryos in vitro. Those methods that are changing most rapidly or that have an important influence on the capability to maintain biological diversity are: (1) Embryo Culture (2) Embryo Storage (3) In Vitro Fertilization (4) Embryo Freezing (5) Embryo Micromanipulation (6) Ovum Maturation (7) Embryo Sexing.

By combining two or more of these methods, it can be predicted that the reproduction of wild and domestic animals will soon become an engineered process. It may ultimately become possible to "design" animals to fulfill specific needs of human societies. However, to prevent the irrevocable loss of existing genetic information, it is recommended that a bank of frozen animal embryos be established under the auspices of the United States Department of Agriculture. Owners of valuable animals, both wild and domestic, would "deposit" a small number of embryos in the bank. Such a bank could be drawn on in the future to recover specific genes that might have been lost from living animals.

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Appendix I

The following is a list of commercially available products listed in the manuscript text as well as the manufacturers and their locations:

Clomid — clomiphene citrate

Merrell-Dow Pharmaceutical Company, Inc.
Cincinnati, Ohio

FSH — follicle stimulating hormone

Burns Biotec
Oakland, California

GnRH — gonadotropin releasing hormone

Sigma Chemical Company
St. Louis, Missouri

Lutalyse — prostaglandin F_2 alpha for use in bovids (dinoprost tromethamine)

Upjohn Company
Kalamazoo, Michigan

Estrumate — synthetic prostaglandin F_2 alpha (cloprostenol sodium)

Haver-Lockhart
Bayvet Division Miles Laboratories, Inc.
Shawnee, Kansas

Appendix II

Below is a list of animals produced through successful artificial insemination.

Non-Domestic Mammals

Addax*
Guanaco
Llama
Blackbuck*
Bighorn Sheep
Brown Brocket Deer
Reindeer
Red Deer
Speke's Gazelle (Boever et al., 1981)
Giant Panda*
Ferret
Fox*
Wolf* (Seager et al., 1975)
Persian Leopard (Dresser et al., 1982a)
Puma (Moore et al., 1981a; Moore et al., 1981b)
Macaca sp.
Papio sp. (Kraemer et al., 1972)
Squirrel Monkey
Chimpanzee* (Hardin et al., 1975; Martin et al., 1978)
Gorilla* (Douglass and Gould, 1980)

Birds

Cranes (several species) (Gee et al., 1978)
Albino Cockatiel
Raptors - Peregrine falcon (Boyd, 1978)
Waterfowl
—ducks
Pheasants

Reptiles

Tortoises*
Alligators

*Frozen semen